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OF THE SIXTEENTH BIENNIAL CONGRESS OF THE INTERNATIONAL SOCIETY FOR ANIMAL CLINICAL PATHOLOGY

JUNE 25–29, 2014
UNIVERSITY OF COPENHAGEN,
FACULTY OF HEALTH AND MEDICAL SCIENCES
COPENHAGEN, DENMARK

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The 16th Biennial Congress of the International Society for Animal Clinical Pathology

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Welcome from the organising committee of the 16th Biennial Congress of the International Society for Animal Clinical Pathology

Dear colleagues,

It is a great pleasure for us to be able to welcome you to the 16th Biennial Congress of the International Society for Animal Clinical Pathology here in Copenhagen.
Due to the enthusiastic support from the scientific committee, the generosity of all our sponsors and the positive feedback from many delegates, it has been a genuine pleasure for us to organise this meeting, which will host delegates and exhibitors from 35 countries representing 5 continents. This has been made possible only through generous funding from our commercial sponsors, The Carlsberg Foundation and The Abildgaard Foundation.
The 16th Biennial Congress of the International Society for Animal Clinical Pathology focuses on "The Frontiers of Veterinary Laboratory Medicine". Our goals have been to identify pioneering speakers and make room for many short research communications to give delegates an insight into the directions into which our field is moving, and – similarly important – to facilitate personal relationsships across the globe with the purpose of strengthening social and professional networks.
We are looking very much forward to hosting you all over the next 4 days.

Yours sincerely,

Mads Kjelgaard-Hansen, chairman

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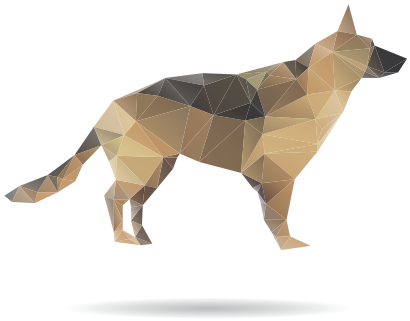

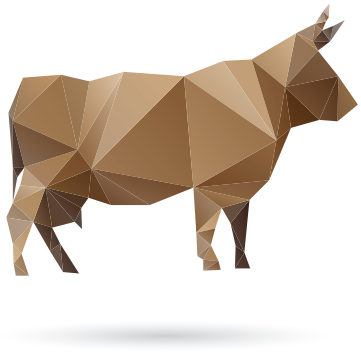

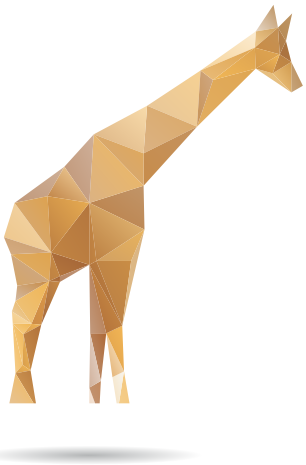

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Invited presentations

POLYPHOSPHATE: AN ANCIENT MOLECULE THAT LINKS HEMOSTASIS AND INFLAMMATION

***S. Smith**, Roger Adams Laboratory, Urbana, IL, USA. E-mail: sasmith6@uiuc.edu*

Inorganic polyphosphate (polyP) is widespread throughout biology, although its contributions to mammalian physiology have only recently been explored. Recently reported roles for polyP in mammalian biology include cell proliferation, angiogenesis, bone mineralization, energy metabolism and tumor metastasis. It was recently discovered that dense granules of human platelets contain abundant levels of polyP, and that polyP is efficiently secreted upon platelet activation. It is strongly procoagulant, leading to shortening of plasma clotting times and rendering clots more resistant to fibrinolysis. PolyP is an extremely potent activator of the contact pathway, accelerates the activation of factors V and XI, abrogates the anticoagulant activity of tissue factor pathway inhibitor, and enhances the thickness of fibrin fibrils. PolyP can be both strongly prothrombotic and pro-inflammatory in vivo, the latter as a consequence of bradykinin release following activation of the contact pathway. Recent work has also provided insight into the pro-inflammatory effects of polyP, showing additional effects on the complement pathway and histones, as well influences on responses by endothelial cells, platelets, and mast cells. This work has likely only scratched the surface of the extensive functions of polyP in innate immunity, inflammation, and tumorogenesis.

COMPARATIVE BIOCHEMISTRY OF METABOLIC DISORDERS IN DOGS AND CATS

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Abstract

Prevalence of obesity and diabetes mellitus (DM) as metabolic disorders has increased in dogs and cats as in human. Cats have unique characteristics in metabolism that 1) hepatic glucokinase (GK) activity is lacking, 2) lower activity of insulin signaling pathway in insulin sensitive tissues and 3) lower plasma adiponectin concentrations than in dogs. Owing to their unique characteristics in glucose and lipid metabolism, cats tend to become obesity accompanying insulin resistance compared to dogs. Obesity (ectopic lipid accumulation) is a risk factor of multiple metabolic disorders including type 2 DM (T2DM), cardiovascular diseases and fatty liver dysfunction in dogs and cats. Obesity induces aberration of adipokine (adipocytokine) secretion and hyperlipidemia leading to lipotoxicity, which causes pancreatic tissue injury and insulin resistance. Ten to 15% of clinically healthy dogs and cats are diagnosed as metabolic syndrome (MS) by the newly established criteria. Prevention of MS reduces occurrence of severe metabolic disorders like T2DM, cardiovascular diseases and fatty liver. Early diagnosis of MS is very important also in dogs and cats. In particular for cats, as therapeutic exercise is difficult, developments of accurate diagnosis for metabolic disorders and anti-obesity supplement and drug are urgent subjects. In recent years, we have tried to develop effective supplements and chemical compounds for obesity in dogs and cats based on the new concept. Studies on comparative biochemistry of glucose and lipid metabolism contribute much to developing of effective treatment for metabolic disorders in dogs and cats.

Introduction

In recent years, prevalence of obesity is rapidly increasing due to drastic changes in lifestyle, particularly eating habits in dogs and cats as in human (Mori et al., 2012). Obesity is closely associated with insulin resistance, which triggers and/or accelerates multiple metabolic disorders including type 2 diabetes mellitus (T2DM), cardiovascular diseases, and fatty liver dysfunction (Arai and Miyazaki, 2014). It is widely known that insulin resistance is caused by chronic and low-grade inflammation in obese adipose tissue (Hotamisligil et al., 1993; Neels and Olefsky, 2006). Cats are known to be apt to become obesity with insulin resistance compared to dogs (Hoenig, 2012). There are significant differences in glucose and lipid metabolism in tissues between dogs and cats, and the differences cause the species specific disorders in energy metabolism in dogs and cats. Type 1 DM (T1DM) (>50% DM cases) appears to the most common form of canine DM, whereas T2DM prevails in cats (80-95% DM cases). Since T2DM is predominant in cats, it stands to reason that insulin resistance is more frequently observed or encountered in cats than in dogs (Rand et al., 2004).

In the present study, I view on the differences in onset mechanisms of metabolic disorders and treatment strategy for metabolic disorders based on the differences in characteristics of energy metabolism (comparative biochemistry for pathogenesis) between dogs and cats.

Comparative study in glucose and lipid metabolism in dogs and cats

1) Glucose metabolism in dogs and cats

Feline livers lack activities and mRNA expression of glucokinase (GK), one of the rate-limiting enzymes in glycolysis, with high Km value for glucose (Tanaka et al., 2005) (Fig. 1 & Fig. 2). Gluconeogenesis enzyme activities in feline liver are significantly higher than those in canine (Washizu et al., 1999). So, feline livers can intake less amount of glucose than canine, and feline livers are considered to be a glucose-producing organ. Plasma metabolite and hormone concentrations and hepatic metabolic enzyme activities in clinically healthy dogs and cats are shown in Table 1 (Tanaka et al., 2005; Mori et al., 2010). There were no significant differences in plasma glucose and lipid concentrations between dogs and cats, whereas plasma adiponectin concentrations in cats were significantly lower than those in dogs. Activities of FK, PK, LDH, G6PD, FBPase in cytosolic fraction and G6Pase in mitochondrial fraction in feline liver were significantly higher than those in canine liver. Feline liver has lower activities of glucose uptake and usage (glycolysis) and higher activities of glucose producing (gluconeogenesis) than canine liver.

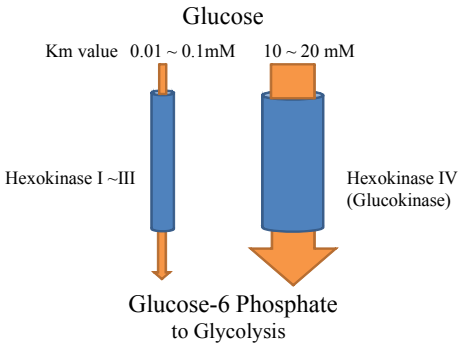

Fig. 1: Km value for glucose of Glucokinase

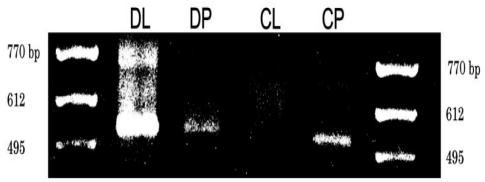

Fig. 2: Analysis of GK mRNA with RT-PCR in canine and feline liver and pancreas. DL, canine liver; DP, canine pancreas; CL, feline liver; CP feline pancreas. DNA size markers of 770, 612 and 495 base pairs.

Table 1: Plasma metabolite and insulin concentrations and activities of metabolic enzymes in liver of clinically healthy dogs and cats

Six male beagle dogs (3-5 years old) and six male mixed-breed cats (4-8 years old) maintained for research in our laboratory were used in this study. Values are presented as mean±SD. ND, not detected

	Dogs (n=6)	**Cats (n=6)**
Plasma Glucose (mg/dl)	90±10	105±18
Triglyceride (mg/dl)	40±10	50±11
Total cholesterol (mg/dl)	143±24	128±24
Insulin (ng/ml)	1.1±0.2	2.0±0.3*
Adiponectin (μg/ml)	38±6	9±2*
Liver enzyme activities (nmol/min pr mg protein)		
HK	4±1	10±2*
GK	13±4	ND
PK	56±6	88±22*
FK	14±2	19±3*
LDH	1571±515	2539±596*
G6PD	11±2	31±4*
FBPase	41±10	102±18*
G6Pase	197±33	345±55*

*Significantly different ($p<0.05$) from the dogs values.

Abbreviations: HK, hexokinase; GK, glucokinase; PK, pyruvate kinase; FK, fructokinase; LDH, lactate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; FBPase, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase.

GK and PK are rate-limiting enzymes for glycolysis and G6Pase and FBPase are rate-limiting enzymes for gluconeogenesis.

2) Lipid metabolism in dogs and cats

A common sign of obesity is hyperlipidemia, which refers to increased concentrations of lipid in circulating blood. Aberrations in cholesterol and/or triglyceride levels can result from high fat diets and/or diseases associated with obesity in dogs and cats (Bauer, 2004). Lipoproteins are believed to play important roles in energy and lipid metabolism of animals, and can reflect metabolic changes. Dog and cats are classified into HDL dominant mammals (Chapman 1986) differing from human as LDL dominant mammals with 4 major classes of lipoproteins: chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density-lipoprotein (HDL). Obesity in dogs has been previously reported to result in aberration to cholesterol lipoprotein fraction profiles (Jerico et al., 2009). Obese dog and cat show different cholesterol lipoprotein fraction profiles differing from healthy animals as Fig. 3 (Mori et al., 2011). In obese animals, activity of cholesterol ester transfer protein (CETP), which transfer cholesteryl ester from HDL-cholesterol to LDL-cholesterol, was elevated significantly compared to healthy animal (Mori et al., 2011). Obese cats showed significant increase in plasma non-esterified fatty acid (NEFA) and LDL cholesterol fractions at early stage of obesity. Elevated NEFAs cause insulin resistance and show toxic effect on β cell proliferation (Pascoe et al., 2012).

In our previous study (Lee et al., 2011), expressions of sterol regulatory element-binding protein-1c (SREBP-1c), and downstream lipogenic genes, such as ATP citrate lyase (ACL) and fatty acid synthase (FAS), in abdominal adipose and liver tissues were determined in diet-induced overweight cats. In the overweight cats, differences in lipogenic gene expression profile between omental and subcutaneous adipose tissue was noted. In

omental adipose tissue, SREBP-1c and FAS expression was significantly higher, whereas ACL expression was significantly lower in overweight as compared to control cats. Recent studies in dogs and humans have reported that mRNA levels of SREBP-1c do not coincide with the changes in adipose lipogenic gene expression. It may be possible that the active, nuclear form of SREBP-1c is increased in an overweight state despite the observed decrease in mRNA expression levels.

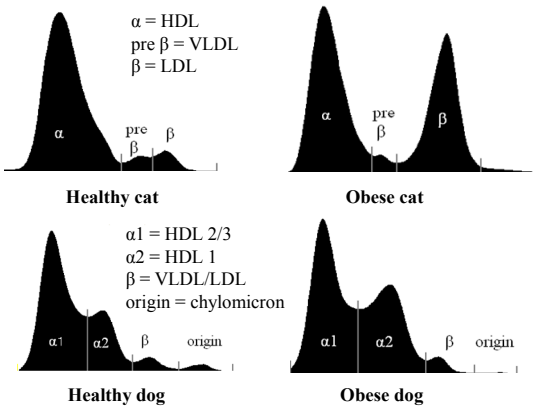

Fig. 3 Electrophoretic patterns of plasma lipoprotein cholesterol of dog and cat

3) Insulin signaling system

A major action of insulin is the regulation of metabolic pathways of protein, glycogen, and fatty acid synthesis. Insulin binding to the extracellular α-subunit of its receptor leads to the recruitment and tyrosine phosphorylation of intracellular substrates such as insulin receptor substrate (IRS) 1-4. Phosphorylations on the IRS proteins bind the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-K). IRS-1, IRS-2 and PI3-K are important downstream players of insulin, and have been implicated with the incidence of insulin resistance and diabetes. Species comparison of insulin signaling genes mRNA levels by q-RT-PCR in insulin sensitive tissues are shown in Fig. 4 (Mori et al., 2009). IRS-1 expression was similar in muscle and fat between cats and dogs. When IRS-2 expression was examined, all canine tissues had a significant increase in expression versus feline tissues except for fat where it was similar. When PI3-K expression was compared, the results positively correlated with IRS-1 and/or IRS-2 expression profile thus confirming that IRS-1 and IRS-2 can activate PI3-K. Since IRS proteins and PI3-K show critical link to insulin signaling, changes in IRS-1 and/or IRS-2 expression should mirrored by changes to PI3-K expression. PI3-K expression is generally higher in canine tissues than feline tissues. Low IRS-1 and IRS-2 protein levels have been found in patients with obesity induced insulin resistance and type 2 DM (T2DM) and in animal models. IRS-1 deficient mice are growth-retarded and show skeletal muscle insulin resistance but do not develop diabetes because the hyperinsulinemia associated with the β cell hyperplasia in these mice efficiently compensates for insulin resistance, whereas IRS-2 deficient mice develop diabetes, presumably due to inadequate β cell proliferation combined with insulin resistance, and tend to become obesity with insulin resistance (Hashimoto et al., 2009). Cats with low expression of IRS-2 mRNA tend to become obese more frequently compared to dogs.

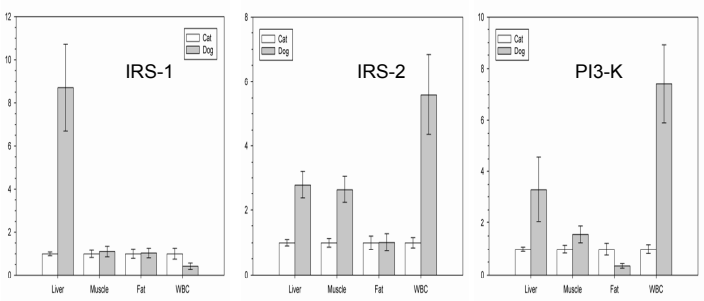

Fig. 4 Comparison of insulin signaling genes in insulin sensitive tissues in dogs and cats

Onset mechanism of obesity and diabetes in dogs and cats

Recent evidence suggests that reduced lipid storage in obese adipose tissue contributes to ectopic lipid accumulation in non-obese tissues such as the liver, skeletal muscle, and pancreas, where lipotoxicity impairs their metabolic functions (DeFronzo, 2010; Suganami et al., 2012).

Fig. 5 summarizes onset mechanism of obesity followed by diabetes in animals. Excess calorie and physical inactivity induce hyperglycemia followed by increased insulin secretion, which accelerates fatty acid synthesis via activation of transcriptional factor, SREBP-1c etc. Acceleration of fatty acid synthesis induces ectopic lipid accumulation, and visceral fat accumulation is increased (obesity state). Aberration of adipokine (adipocytokine) secretion is exhibited remarkably and unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity critically contributes to the development of any aspects of the metabolic syndrome (insulin resistance). Adiponectin exerts antidiabetic effects on muscles and liver through AMP-activated protein kinase (AMPK) activation and antiatherosclerotic effects by inhibiting monocyte adhesion to endotherial cells and lipid accumulation into macrophages. Decreased adiponectin secretion and increased inflammatory cytokines and NEFA secretion from swelling adipose tissue deteriorate insulin resistance in obese animals. Then hyperglycemia, hyperinsulinemia and accelerated lipid synthesis are maintained and hyper-secretion of insulin force excessively heavy work on pancreatic β cells. In over functional pancreatic islets, β-oxidation of fatty acid is accelerated resulting in excess amount of reactive oxygen species (ROS) production, which induces ROS stress leading to mitochondrial dysfunction and apoptosis of β-cells with low scavenging activity of ROS (tissue injury). Inflammatory cytokines from corpulent adipocytes appear to participate in destruction of islets β cells leading to T1DM. The toxic effect of NEFA is mediated via formation of ceramide, increased nitric oxide production and activation of the apoptotic mitochondrial pathway. A series of inflammatory reaction appear to have important roles in the β cell destruction process in dogs and cats with insulin resistance. Lipid metabolism abnormality (lipotoxicity) with increased plasma NEFA and LDL cholesterol and decreased plasma adiponectin levels contribute to lead onset of DM in animals with insulin resistance.

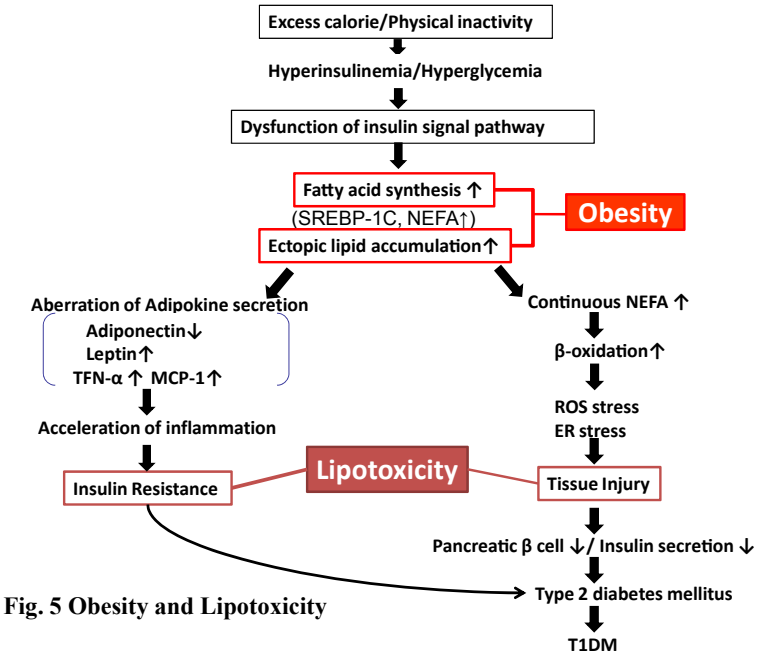

Fig. 5 Obesity and Lipotoxicity

Importance of prevention for obesity in animals

1) Metabolic syndrome criteria

For animals, radical cure for serious metabolic diseases like DM is very difficult. The most effective treatment is prevention for metabolic disorders. To prevent the serious diseases, early diagnosis is very important. In particular for cats, as therapeutic exercise is difficult, developments of accurate early diagnosis for metabolic disorders and anti-obesity supplement and drug are urgent subject. Obesity is a risk factor for multiple metabolic disorders including T2DM, cardiovascular diseases and fatty liver dysfunction, and the metabolic disorders worsen gradually as follows:

Obesity → Hyperlipidemia → Insulin resistance (metabolic syndrome, MS)
→ Serious disorders (diabetes, kidney dysfunction etc.)

Prevention of MS reduces prevalence of DM in human. We tried to establish the temporary criteria of MS for dogs and cats (Table 2). In our research with the temporary criteria, 10 to 15% of clinically healthy dogs and cats are diagnosed as MS (Mori et al., 2012). Dogs and cats diagnosed as MS showed high NEFA and low adiponectin concentrations in the plasma.

Table 2 Temporary criteria for metabolic syndrome diagnosis of dogs and cats	
Dogs	**Cats**
Central obesity (defined as over 10% increase in normal BW or BCS>3.0) and any two of the following 3 factors	
Plasma GLU ≥120mg/dL	Plasma GLU≥120mg/dL
Hyperlipidemic condition, diagnosed with any two of the following factors, TG ≥165mg/dL, TC≥200mg/dL, NEFA≥1.5mEq/L	TG≥165mg/dL and/or TC≥180mg/dL
ALT≥100IU/L	ALT≥100IU/L
Abbreviations: GLU, glucose; TG, triglyceride; TC, total cholesterol; NEFA, non-esterified fatty acids; ALT, alanine aminotransferase	

2) Treatments for obesity

It has recently become widely accepted that obesity is characterized by chronic low-grade inflammation of adipose tissue that predisposed affected individuals to insulin resistance (Gauthier and Ruderman, 2010). Appropriate treatment for obesity appears to be a key factor to prevent the progression to severe metabolic disorders. Furthermore, it has been clarified that oxidized-LDL strongly induces oxidative stress leading to metabolic disorders (Van der Zwan et al., 2010). We have tried to develop the new supplement, diet and drug for obesity based on the new concept as follows:

- Supplement: flavonoid extracted from root of the *Glycyrrhiza* species; depress fatty acid synthesis, acceleration of lipolysis in tissues and depress oxidative stress (Nakagawa et al., 2004)
- Diet: weight loss diet for dogs; low fat, low calorie, high crude fiber, higher protein
- Chemical compound (drug): apoptosis inhibitor of macrophage (AIM) in both types of immune response; depress fatty acid synthesis and acceleration of lipolysis in liver, kidney and adipose tissue (Arai and Miyazaki, 2014)

Studies on comparative biochemistry of glucose and lipid metabolism contribute much to developing of effective treatment for metabolic disorders in dogs and cats.

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NON-INVASIVE ENDOCRINE MONITORING: POSSIBILITIES AND LIMITATIONS

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Over the last three decades, studies have illustrated the potential for non-invasive monitoring of reproductive and stress steroid hormone metabolites. These techniques have substantially enhanced our understanding of the physiology of a number of animal species. Various techniques have been applied to a wide range of research questions studying wildlife, domestic and laboratory species. The techniques are widely accepted, and in many studies have become a substitute for analysing steroid hormones in serum/plasma. Although measurement may appear straightforward, it has been recognized that non-invasive assays require far more extensive validation than the corresponding plasma assays. Because of species-specific differences in steroid metabolism in even closely related species, careful validation of assay methods is necessary in order to generate meaningful and accurate results. Validation in reproductive studies is rather straight forward, whereas studying the stress response usually is based on an ACTH challenge test. Excreted steroids are heavily metabolized; therefore specific assays like those typically used for the analysis of hormones in blood samples should be replaced by assay with high cross-reactions against a certain group of steroids (group-specific assays). Other concerns are extraction techniques, storage and stability of faecal metabolites, determination of faecal immuno-reactive steroid metabolites by HPLC, gut transit time, diurnal and seasonal variations, etc. As currently researchers are applying different techniques, results between studies are only comparable in their physiologic outcome, but usually not in absolute metabolite concentrations. Beside some limitations of use, endocrine monitoring has enormous potential for how we manage wildlife species both in situ and ex situ.

Several reviews on non-invasive monitoring of hormones can be found in a special issue of Vet Med Austria **100 (2013).**

SALIVA IN VETERINARY SCIENCE: THE PRESENT AND THE FUTURE

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Saliva renders a portal through which systemic animal health could be monitored, since salivary components include both locally synthesized and systemic proteins that could act as biomarkers. There are several advantages for the use of saliva instead of serum as a diagnostic fluid in veterinary. Firstly, saliva testing has facilitated the safe, efficient and low-cost collection of large numbers of diagnostic samples. Additionally, the use of saliva samples would provide a practical method for repeated sampling without causing stress to the animals and could be especially useful in species such as pigs where blood sampling is technically difficult.

In our presentation we will try to provide updated information about some technical issues regarding saliva sampling and processing. Also we will outline our experience with analytes that can be measured in saliva such as:

-Biomarkers of inflammation
-Biomarkers of stress

In addition we will comment the last advances made in proteomics in saliva.

Overall we expect that this presentation will provide an overview of the potential application of saliva as a non-invasive diagnostic sample. As well it could stimulate the international collaborations in the development of future research projects in order to gain knowledge in this field.

METABOLOMICS, MARKERS AND MECHANISMS.

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Metabolomics is one of the more recent technologies to enter the 'omics era. Metabolomics is the comprehensive characterization of small molecules in a sample set and is most often combined with multivariate statistics for data mining. The instrumentation used may be any kind of spectrometry including nuclear magnetic resonance (NMR) and time-of flight mass spectrometry (TOF-MS), the latter often with a liquid (LC) or gas (GC) chromatography step to precede spectral analysis. There is no limit to the sample types that it may be technically feasible to profile by metabolomics but an extraction step is often necessary. Metabolomics has a major application in exploration, where we seek to map all major differences between samples representing different treatments or observational factors. For example this methodology was used to map similarities and differences between body fluids and organs in pigs as a reference for further study. We have also applied it extensively to look for effects of different dietary factors and especially for finding new and better markers of dietary exposures. Another central application is for finding markers of dietary effects that may relate to subsequent risk of disease. For instance the sequence of metabolic changes taking place postprandially may differ with risk of pending metabolic disease. Mapping the metabolic changes helps in elucidating mechanisms in nutritional disease but it is equally useful for characterizing disease states and any other phenotypic characteristics. We and others have applied it for finding markers or mechanisms in cancers, cardiovascular disease and diabetes but studies on infectious and genetic disease have also emerged recently. The data-driven approach inherent in these applications has the primary goal of creating better and more informed hypotheses for further research. Examples will be given of how metabolic profiling has led to new testable hypothesis related to disease mechanisms.

PLAYING WITH THE PAST – THE GEOGENETICS APPROACH TO EVOLUTION, ANTHROPOLOGY AND BIODIVERSITY

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Describing, and better still, developing an understanding of, the past remains one of the key fascinations for many biologists. Although many disciplines achieve this through exploiting fossil or similarly ancient biological material, geneticists have predominantly been limited to drawing inferences on the past using patterns observe among modern day nucleic acid sequences. Although often such approaches make remarkable discoveries, they suffer from a single key limitation - they are unable to account for the effects of lost genetic diversity.

The Centre for GeoGenetics is a Danish Basic Research Foundation Centre of Excellence, that was created under a core premise - that considerable amounts of genetic information can be extracted from historic, archaeological and even palaeontological biological material, and given recent developments in DNA sequencing and bioinformatic techniques, we have entered an era where such 'ancient DNA' can lead to discoveries that truly shift paradigms. In this talk I draw on examples of ancient DNA based research undertaken within the Centre relating to evolution, anthropology and biodiversity, in order to highlight just how much information such samples contain. I furthermore discuss the limitations fo the field, and thus outine suggestions of some key research questions that we might anticipate answers to within the next decade.

THE CLINICAL LABORATORIAN AS AN AGENT OF TRANSFORMATIONAL CHANGE

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Everything revolves around the job that we do as animal clinical pathologists. Research, development, clinical service, and education are all integral subunits of successful projects and clinical applications. Much of what we do is seamless to those who depend on us, but we are there to bridge the gap between research and reality and to provide a key element of unification to pathology and veterinary medicine. To an animal clinical pathologist, updates to equipment, reformulations of assays, and new guidelines for use and interpretation of tests are a routine part of our work. It is our responsibility to keep forward momentum in all these areas to best serve our clients – be it researchers or clinicians. In many times during our career, we have the opportunity to be the catalyst for transformational change. That is, to get those who we serve to reinvent the way they think about clinical pathology and to question their satisfaction with the status quo. This process, of course, is quite contrary to the part of human nature that resists change. In this presentation, we will relate our own experiences and discuss some ideas that we, as individuals and as a team, can use in our own campaigns for transformation.

Keynote presentations

PARASITOLOGICAL INFECTIONS AND THE INFLAMMATORY PROCESS

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No studies have been reported regarding nematode parasite infections in dogs in Brazil and specific acute phase protein changes. The giant kidney *Dioctophyme renale* is one of the largest parasite nematodes infecting different animal species, including in humans, with female adult worms measuring up to 100 cm length and 1 cm diameter. It is found in the renal parenchyma or free in the abdominal cavity. The life cycle is complex and requires an aquatic oligochete or annelid as intermediate host and a paratenic host (fish or frog) (Anderson, 2000). The literature is limited mostly on case reports on dogs and wild carnivores and mustelids in Brazil such as the maned-wolf (*Chrysocyon brachyurus*), crab-eating fox (*Cerdocyon thous*), coatis (*Nasua nasua*) and the lesser grison (*Galictis cuja*). There are also reports in the literature of adult worms found free in the abdominal cavity of a domestic cat and of a capuchin monkey (*Cebus apella*). Although clinical diagnosis methods are used to support the detection of this parasite such as imaging techniques and the observation of the *D. renale* ova in the urine and the suggested treatment is surgical with nephrectomy or nephrotomy, there are no reports regarding the inflammatory reaction caused by the infection or on systematic monitoring of recovery after surgery. Recently, we investigated an outbreak of this parasite infection in dogs in São Paulo State, southeastern Brazil. Fourteen dogs had *D. renale* eggs in the urine sediment and all the animals were submitted to nephrectomy. During exploratory laparotomy to perform the nephrectomy, in one case only one adult D. renale worm was found free in the abdominal cavity. The other animals had adult worms only in the right kidney and free in the abdominal cavity, in numbers of 1 to 14 females and males worms. Blood samples were obtained from all the dogs before surgery and in 14 different time points after nephrectomy. Three positive acute phase proteins (CRP, SAA and Haptoglobin) and one negative acute phase protein (albumin) were measured in serum aliquots from all the animals in the 15 time points. The hemogram, total protein, urea and creatinine were also performed in all time points from all dogs.

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HEMOSTATIC CHANGES REVISITED IN VIRULENT CANINE BABESIOSIS

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Tick-borne intra-erythrocytic protozoan parasites belonging to the species *Babesia* constitute a significant cause of clinical disease in dogs worldwide. In South Africa, babesiosis is caused predominantly by *Babesia rossi*, the more virulent strain, and has been identified as the most common cause of morbidity and mortality among dogs. The severity of virulent canine babesiosis has been reported to be due to an exuberant and ineffective immune response that sometimes results in lethal collateral organ damage. It seems clear that disruption in normal hemostasis may be crucially involved in this complex pathogenesis. Current investigations are based on the hypothesis that dysregulation of hemostasis play a central role in the pathogenesis of virulent canine babesiosis and that changes in hemostasis profiles will predict clinical complication and outcome in dogs admitted to the hospital for babesiosis.

Severe thrombocytopenia, with platelet counts (PLT) often less than 25×10^9/L, is a consistent finding in virulent canine babesiosis; however, clinical hemorrhage is not a frequent finding in the disease. One study[1], using thromboelastography (TEG), reported normal TEG variables in the face of severe thrombocytopenia for dogs with uncomplicated babesiosis (PLT=20×10^9/L) compared to healthy control dogs (PLT=375×10^9/L), indicating the presence of a relative hypercoagulable state compared to what is normally seen in dogs with such extremely low platelet counts. A significant positive association was reported between PLT and MA; however, no significant associations were reported between hematocrit or fibrinogen concentration and any of the TEG variables. The presence of a hypercoagulable state was also reported in a study[2] that evaluated the differences in plasma coagulation factor activity, anticoagulant activity and D-dimer concentration between survivors and non-survivors in dogs with complicated virulent babesiosis. The study reported that mortality was associated with a more severe consumptive coagulopathy characterized by procoagulant activation, inhibitor consumption and fibrinolysis activation without the presence of gross hemorrhage, thus fulfilling the requirements of non-overt DIC.

Blood platelets are key elements linking the processes of hemostasis, inflammation and tissue injury. Platelet activation is a process during which platelets change shape, release the contents of the alpha and dense granules, and express receptors on their surfaces to facilitate their aggregation. Platelet indices, reported to be surrogate markers for platelet activation, have shown significant differences in virulent canine babesiosis. Mean platelet volume (MPV) and mean platelet mass (MPM) was significantly increased in infected dogs compared to healthy controls, with no differences between survivors and non-survivors. Surprisingly, the mean platelet component (MPC), a measure of platelet granularity and thus activation status, was not significantly decreased in the infected dogs. Large platelets may, therefore, play a significant procoagulant role in the lack of clinical hemorrhage, despite severe thrombocytopenia, but does not appear to play a role in disease severity or outcome.[3]

Evaluation of platelet-leukocyte heteroaggregation, using flow cytometry, reported significant differences between survivors and non-survivors.[4] *Babesia*-infected dogs,

specifically dogs that survived, had a significantly up-regulated response with regards to platelet activation and platelet-monocyte heteroaggregation. Moreover, the dogs which died showed no difference compared to the healthy controls. These findings may indicate altered α-granule content, as has been suggested in humans with sepsis, or platelet "exhaustion" secondary to the severe systemic inflammation present, resulting in immune "paralysis" and possible death.

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CURRENT STATUS AND CHALLENGES OF ANIMAL CLINICAL PATHOLOGY IN THE MIDDLE EAST WITH SPECIAL REFERENCE TO EGYPT

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Veterinary clinical pathology is one of most important tools of diagnosis of animal diseases. Despite the presence of considerable numbers of Faculties of Veterinary Medicine in the Middle East, especially in Egypt, clinical pathology is usually not considered as a separate science, but is part of pathology education and services. As is well known, clinical pathology has great value in education, research and services. In the Middle East there are major and serious challenges to be overcome. The main problems facing clinical pathology are variation in teaching strategies and curricula between schools. Also, a shortage of facilities and teaching tools accompanied by shortage of staff are considered among the most important difficulties. Financial support, standardization of testing, and accreditation of laboratories are also challenges facing clinical pathology research and services in the Middle East. Clinical pathology testing is used often in Egypt for monitoring toxicologic and pharmacologic research studies in laboratory animals, but most of these tests are done by toxicologists, not by specialists trained in clinical pathology. In spite of the disadvantages that seem to face veterinary clinical pathology, the Middle East is a unique and fascinating region with novel species (like camels) and important animal diseases, especially infectious diseases that provide opportunities for future collaboration and development in clinical pathology research, education, and services.

STATUS REPORT FOR VETERINARY CLINICAL PATHOLOGY IN JAPAN

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Educational requirements for clinical pathology have been established for veterinary licensure in Japan for more than thirty years. Of the sixteen veterinary schools in the country, ten include this training as part of their course requirements for internal medicine while the remaining six schools offer specialized training for students to focus primarily in clinical pathology.

Currently there are approximately 13,500 veterinarians operating 11,000 veterinary clinics with about one third of them located in the Tokyo area. About 70% of clinics have their own hematological and blood chemistry screening and diagnostic equipment in-house. Additionally there are 32 commercial labs that provide veterinarians with diagnostic information including CBC, blood chemistry, hormone assays, allergy tests, genetic tests, lipid metabolism, and cytological and pathological diagnosis. Commercially available genetic tests include the diagnostic test for infectious diseases, drug sensitivity and resistance, clonality of lymphoma, c-kit mutation, microsatellite analysis, and inherited diseases.

There are four clinical pathologists qualified by the ACVCP and five anatomic pathologists qualified by the ACVP in Japan. Only one clinical pathologist among them is a faculty member of a veterinary school and the rest of them are working in commercial laboratories or running their own business. There are three Japanese anatomic pathologists working in the USA including two faculty members of veterinary schools and one company employee. Presently there are four Japanese veterinarians undertaking residency program courses in clinical pathology in the USA. An equivalent qualified residency program and examination has yet to be established in Japan. Hopefully these young veterinarians will be able to return to Japan and help establish a new program for advanced specialized training in clinical pathology in Japan and Asia.

PRESENT STATUS, CHALLENGES AND FUTURE PERSPECTIVES OF THE VETERINARY CLINICAL PATHOLOGY IN CUBA: EXPERIENCES WITH THE CUBAN CATTLE GENOTYPES (HOLSTEIN X ZEBU)

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The objective of this work is to contribute to the popularization of the current state, challenges and future perspectives of the Veterinary Clinical Pathology (VCP) in Cuba, emphasizing the experiences obtained in this field in genotypes of bovine livestock Holstein x zebu. At the end of 1960, VCP was consolidated as a discipline in Cuba; and at the beginning of the 1970s the first texts were published about the principles and methodologies of VCP. The first studies were on the metabolic profile and their relationship with the lactation period, milk production and the fertility of the bovine females Holstein and its breeds with the Zebu and the effect that it has in its breeding level; the metabolic diseases and the influence of the metabolic state on the fertility levels of its bull sires. Nevertheless, the field which was studied much was the mineral profile of the bovine of different breeds, associating it to fertility; in these studies they were carried out determinations of the organic reservations of minerals. In the 1990s VCP was affected by the economic limitations of the country and no further investigations were carried out in this field until the beginning of the years 2000, where the feeding conditions in the Cuban cattle rearing differed substantially with that of the years in which they were carried out the mentioned studies and that without doubts, it would rebound on the different parameters of the metabolic profile. In this stage, anemia caused by iron deficiency was studied in suckling calves and also the hematological profiles of the cows Holstein and its breeds were studied. Our group studied the metabolic state and especially the levels of minerals of the bovine females Holstein x Zebu and its relationship with the reproduction under the new conditions of the Cuban cattle breeding; the most outstanding results demonstrated that a negative correlation was established between the levels of copper in serum in a range of 9.8 ± 1.0 to 14.0 ± 0.9 µmo/L with the reproductive indicators and that the administration of 50 mg of Cu for via parenteral in the bovine females with copper levels in blood of until 14 µmol/L that included values considered normal of (>11.77 µmol/L), this increases its reproductive efficiency. When the serium levels of Zn descend from 15 µmol/L the cows are at risk of developing anestrous, which appears frequently before the values that indicate its deficiency appear (12.62 µmol/L). It was found differences in the hepatic indicators and those of protein metabolism in the ones found in Cuba for the cows Holstein in the 1980s, under other conditions of feeding, management and racial groups. It was studied the mineral profile, protein profile, hematological and acid base profile for several species. Recently it was incorporated the study of the acute phase proteins (APPs) in the canine species and their alterations in parvovirus disease. Up to now, the results have been discussed taking into consideration the reference parameters published internationally, that makes it difficult its correct interpretation because they are several sources of variation in the biochemical indexes used in the VCP; for which our work group at the moment has established the reference parameters for the main indicators of the metabolic profile in several species, adjusted to our conditions. The main challenges and future perspectives that we have are: to determine the appropriate levels of each metabolic indicator so that it can show an appropriate reproductive efficiency, especially in

the bovine females; to study the relationship of the minerals in sanguine serum with those of follicular fluid and those of the oviduct, the oxidative stress and with the quality of the oocytes and embryos in bovine; to deepen in the employment of those APPs in the diagnosis and prognosis of pathological processes in several species and to incorporate new diagnostic tools. To execute these aspirations it will be of great utility the international collaboration and the academic exchange with other institutions to execute combined projects that allow improvement of the infrastructure and the equipment, and the formation of human resources.

CURRENT STATUS OF VETERINARY CLINICAL PATHOLOGY IN THAILAND

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There are 6 Veterinary faculties in Thailand; Chiengmai (northern), Khonkhen (north-eastern), Chulalongkorn, Kasetsart, Mahidol University, and Mahanakorn Technology Institute (central Thailand). About 500 DVMs graduate every year. Veterinary clinical pathology (VCP) in Thailand is mainly included hematology, blood chemistry, cytology and urinalysis. Molecular biotechnology is also performed for species identification of pathogens and forensic investigation. Our faculty has 4 Animal Hospitals (AH) which most cases are dogs and cats.

Hematology are performed both automate cell counter and gold standard manual methods (blood smear examination, packed cell volume, plasma protein, fibrinogen and reticulocyte count). Manual hematology are performed for avian and reptile samples. The most cases of VCP service are hematology and blood chemistry. The blood smear evaluation is the most important for the hematological abnormalities especially blood parasite identification. Despite the advanced technology of rapid immunological tests for blood parasite detection, we still use buffy coat examination, which are cheaper and more definitive diagnosis but they are more time consumption. Due to the tropical country, about 1/4 of dogs submitted to AH were infected with blood parasites [1]. We also found the infections of *Anaplasma platys* in domestic cat [2] and *Hepatozoon* sp in flat-headed cat and Leopard cats [3, 4]. Molecular techniques played a major role in identification of blood parasites in raptors submitted to Kasetsart University Raptor Rehabilitation Unit [5, 6]. We also collaborated with the zoo [7, 8] and reptile farm [9] in hematological studies.

During January 2012-December 2013, there were 8,272 dog samples and 1,252 cat samples submitted to Bangkhen AH for cytology diagnosis. Definitive diagnosis for tumor was 39.14% for dogs and 26.75% for cats. The most prevalence of tumor in dogs was mast cell tumor (14.2%) and in cat was lymphoma (44.8%). Anyhow, in dogs the percentage of lipoma (8.1%), melanoma (3.9%) and adenocarcinoma (3.9%) were remarkable. Private AHs set up their own VCP laboratory. Private VCP laboratory in Bangkok serve small clinics. Most of the other private clinics in the small provinces send the samples to human laboratory. Anyhow, the number of graduate student for VCP in Thailand was very low and we have no residency program for VCP.

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Short oral presentations

DYNAMICS IN SYNOVIAL FLUID CONCENTRATIONS OF SELECTED HAEMOSTATIC BIOMARKERS IN AN EQUINE MODEL OF JOINT INFLAMMATION

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Septic arthritis is a common and potentially devastating disease in horses characterized by severe intra-articular (IA) inflammation and fibrin deposition. Research into the pathogenesis of equine arthritis has almost exclusively focused on inflammation while the role of the haemostasis remains un-investigated. The aim of this study was to characterize the dynamics of selected biomarkers of IA haemostatic and inflammatory responses in horses with experimental lipopolysaccharide (LPS)-induced joint inflammation. LPS (3 ug) was injected into one radiocarpal joint of adult horses (n = 6). Synovial fluid (SF) samples were collected repeatedly before and after (until 144 hours) injection, and total protein (TP), white blood cell (WBC), serum amyloid A (SAA), haptoglobin, iron, fibrinogen, thrombin-antithrombin (TAT) and d-dimer levels were assessed. SF levels of TP, WBC, haptoglobin, fibrinogen and TAT increased 2-4 hours after LPS injection, whereas SAA and d-dimer levels increased at 16 and 144 hours, respectively. In conclusion, equine joint inflammation was accompanied by an IA haemostatic response. Haemostatic and inflammatory responses had dissimilar, but overlapping, kinetics, which suggests simultaneous activation and/or interaction. Human joint disease research focuses increasingly on markers of both haemostasis and inflammation as these systems are intricately linked as part of the innate immune response, and further studies of the pathophysiologic significance of activation of haemostasis in equine joint disease are needed.

RELATIONSHIP BETWEEN FINE NEEDLE ASPIRATION CYTOLOGY IN BOVINE LIVER WITH AND WITHOUT LIPIDOSIS AND BIOCHEMICAL VARIABLES

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Fatty liver is a major metabolic disorder that occurs in dairy cows. It develops when the hepatic uptake of lipids exceeds the oxidation and secretion of lipids by the liver, and excess lipids are stored as triacylglycerol in hepatocytes. Liver fat content can be measured definitively by chemical methods. In addition, the use of fine-needle aspiration (FNA) cytology to detect hepatic lipidosis in cattle has been described. The aim of this study was to evaluate the relationship between FNA cytology and biochemical variables in cows with fatty liver. We collected 62 liver samples (18 healthy; 44 grossly fatty) post mortem in slaughterhouse setting. Samples for lipid (%) determination were obtained using a biopsy needle. FNA samples for cytology were obtained using a 20 g spinal needle, and smears were prepared and air-dried immediately. Blood samples were obtained individually (before slaughtering) to determine serum concentrations of NEFA(mmol/l), BHB(mmol/l) and GLDH(U/l) activity. Scores were assigned for the percentage of hepatocytes with evidence of lipid accumulation using a 0 to 4 point grading scale (not affected to severely affected). A simple regression analysis was done to relate cytology findings with lipid content and biochemical variables. The relationship between degree of lipidosis established by cytology and lipid content was moderate but highly significant (r= 0,4; p= 0,006). There was also a correlation between cytology and NEFA and BHB concentration, but not with GLDH activity. Results suggest that FNA cytology is likely to be an alternative means of diagnosing fatty liver in cattle.

SERUM AMYLOID A (SAA) CONCENTRATIONS IN NEWBORN REINDEER CALVES SERUM DURING FIRST MONTHS OF LIFE AND ITS ASSOCIATION WITH DAILY WEIGHT GAIN AT AGE OF 2 MONTHS

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Serum amyloid A (SAA) is an acute phase protein with several pro- and anti-inflammatory properties. The aim of this study was to find out if SAA concentration in blood serum profile
changes significantly and predicts reindeer calves daily weight gain in first two months of life.
Blood samples were collected from 28 calves (13 female, 15 male) from May to July 2012. Each calf was weighted at birth (average 5.77 +/- 0.67 kg). During 2 months period every calf was weekly weighted and blood sample collected (average 6.25 +/- 1.8 samples per animal).
In total 175 serum samples were collected and analysed. To find if SAA concentration fluctuated significantly in first two months of life random intercept linear regression model was used, where calf was random factor and week fixed factor. To find if SAA concentration at different week of life had effect on calves daily weight gain during first two months of life regression analysis by week were used. Significantly lower SAA concentration was found at weeks 6, 7, and 8 (103.7 +/- 97.3 mg/l; $p < 0.05$) compared to first week concentrations (156.3+/-93.3 mg/l). Higher SAA concentration on first week of life had positive association with daily weight gain on 56-70 days (329 +/- 34 g/d; $p = 0.005$).. In conclusion, these results suggest that some factors causing increase concentration of SAA in first week of life had positive impact to daily weight gain during at least first two months of life.

HAPTOGLOBIN DECREASES IN EQUINE SERUM AFTER INDUCED COLON ISCHEMIA AND REPERFUSION

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Horses with colon torsion require immediate surgery in order to ensure survival of the compromised intestine and the horse. Intestinal inflammation in horses has been shown to cause changes in serum and peritoneal fluid concentrations of serum amyloid A (SAA) and haptoglobin, but there is a lack of information regarding this during early phases of intestinal vascular compromise and inflammation. The aim of this study was to evaluate serum concentrations of SAA and haptoglobin in response to experimentally induced ischemia and reperfusion of the equine colon. Colon ischemia (1hour) and reperfusion (4 hours) were induced by ligation and subsequent release of the ligature of the pelvic flexure in 5 horses under general anesthesia. Jugular venous blood samples were collected before ischemia (T0) and after ischemia (T1), and after1 hour (T2) and 4 hours of reperfusion (T3). Serum haptoglobin decreased significantly ($p=0.002$) from a mean concentration of 1555 mg/L (T0) to 1200 mg/L (T3), whereas concentrations of SAA did not change significantly. The results suggest that a decrease in serum haptoglobin occurs in the early phase of intestinal ischemia and inflammation. The decrease in haptoglobin concentrations may have been caused by local haemolysis induced by vascular compromise, as haptoglobin is known to bind free haemoglobin. This knowledge can be useful when evaluating the severity of the intestinal and vascular damage in horses with colon torsion. But further studies are needed to understand the pathogenesis of the observed haptoglobin response during colonic ischemia and reperfusion.

DETECTION OF INFLAMMATORY PROTEINS IN EQUINE SALIVA BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Methods for non-invasive sampling for monitoring health and disease in animals have attracted increasing interest over the last years. Use of saliva for monitoring purposes in horses would represent an advantage over blood, as the samples could be obtained with little or no stress to the horse, and – on the account of the ease of sampling – horse owners could perform the sampling themselves to facilitate for example monitoring of disease. Increased concentrations of acute phase proteins (APPs) have been detected in humans, pigs and dogs suffering from systemic inflammation, but the composition of equine saliva is very incompletely uderstood. The objective of the study was to assess global expression of proteins in equine saliva by liquid chromatography tandem mass spectrometry (LC-MS/MS). Saliva was obtained from horses (n = 7) suffering from systemic inflammatory disease and horses without inflammation (n = 6). Tryptic peptides from saliva were analyzed by LC-MS/MS, and 195 unique proteins were identified; 57 of these were only detected in saliva from horses with systemic inflammation. Among the differentially expressed proteins were several APPs such as serum amyloid A, fibrinogen, haptoglobin, and alpha1-acid glycoprotein. The study is the first to describe detection of inflammatory proteins in equine saliva. Overall, detected proteins were similar to those described in saliva from cattle, small ruminants and pigs. Detection of APPs in saliva from horses with systemic inflammation suggests that saliva may be used for non-invasive disease monitoring in horses similar to what has been described for humans, pigs and dogs.

ACUTE PHASE PROTEINS PROFILE IN MILK FROM A DAIRY FARM WEST OF SCOTLAND

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The profile of major bovine acute phase protein (APP), haptoglobin (Hp) was determined in milk from a small holder dairy farm West of Scotland, to confirm whether values correlated with the more commonly utilized indicator of udder health, somatic cell counts (SCC). An in house, newly developed enzyme linked immunosorbent assay for measurement of milk Hp was used for the verification of Hp profile in composite milk (n=54) of the Holstein cows. Quarter milk (n=149) were also assayed for Hp. Composite/cow SCC, stage of lactation (early, mid, late or drying period), parity (lactational number) and milk yield (Kg/cow) data were obtained from farm routine milk quality records and used to ascertain if any correlations existed with Hp.

Quarter milk Hp had median value of 0.36µg/ml, and a range of 0.02-42 µg/ml with 92%of samples having Hp concentration below 2µg/ml. Composite milk Hp ranged from 0.02-35µg/ml with a median of 0.40µg/ml; significant correlations were found only between SCC and Hp ($P<0.05$) as well as parity and Hp ($P<0.05$). Moreover when SCC was categorized into high (>200000 cells/ml) and low (<200000cells/ml), the correlation with Hp was significant at $P<0.01$.

In conclusion, this study demonstrated the potential usefulness of the APP Hp as a substitute or complement to SCC in detecting udder infections, while observing a possible confounding factor such as parity, which should be taken into consideration when confirming diagnosis.

SAA CONCENTRATION IN THE CSF OF DOGS WITH DIFFERENT NEUROLOGIC DISORDERS

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In this preliminary study, serum amyloid A (SAA) was measured in the cerebrospinal fluid (CSF) of dogs with idiopathic epilepsy (n=3), distemper (n=4) and necrotizing meningoencephalitis - NME (n=2) i.e. non inflammatory, viral and idiopathic inflammatory disease of central nervous system, in aim to test its usefulness as a biomarker of neurologic diseases. Control group (n=3) consisted of dogs without history of neurologic diseases, euthanized due to joint problems. Diagnosis of neurological diseases has been done on clinical and neurological examination, microbiology, serology and histopathology. CSF was collected at presentation from cerebellomedullary cistern, and physical characteristics, cytology analysis, protein content, lactate-dehydrogenase (LDH) and creatin-kinase (CK) activity was determined using standard laboratory procedures. SAA was determined using ELISA test kit (Tridelta, Irland). Dogs with idiopathic epilepsy had three times higher, while NME and distemper positive dogs had 15 to 400 times (median=200 times) higher SAA concentration than control group of dogs. No difference between SAA concentrations was found between distemper and NME CSF samples. Protein content and cell number were not related to SAA concentration, while LDH and CK increased 10 times above referent levels only in one case of NME. Despite small number of samples, we could formulate to work hypothesis: 1) modestly increased level of CSF SAA in cases of idiopathic epilepsy could point to low level inflammatory stimulus that should be exploited in the future to define its pathogenesis and 2) CSF SAA is a sensitive biomarker and could be used to monitor inflammatory CNS disease.

MOLECULAR CHANGES ASSOCIATED WITH THE DEVELOPMENT OF RESISTANCE TO IMATINIB IN AN IMATINIB-SENSITIVE CANINE MAST CELL TUMOR CELL LINE

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Imatinib has potent anti-tumor activity against canine mast cell tumor (MCT) carrying c-kit mutation. Although the tumors initially respond well to imatinib, they eventually develop resistance. We established imatinib-resistant cell lines to clarify the mechanisms underlying the imatinib resistance, and molecular changes that confer the resistance were investigated.

An imatinib-sensitive MCT cell line, VI-MC, carrying a c-kit c.1523A>T was exposed to increasing concentrations of imatinib and two sublines that are able to grow under the presence of 1 μM (rVI-MC1 cells) and 10 μM (rVI-MC10 cells) of imatinib were obtained.

These cell lines had a second missense mutation (c.2443G>C) in c-kit. The mutant KIT harboring c.2443G>C showed ligand-independent phosphorylation that was not suppressed by imatinib. Despite of carrying a same second mutation, tolerability against imatinib was different between rVI-MC1 and rVI-MC10 cells. We thus examined the difference of phosphorylation status of KIT downstream signal proteins and p-glycoprotein activity between these two cell lines. ERK was constitutively phosphorylated in both cell lines. This phosphorylation was suppressed by 10 μM of imatinib in rVI-MC1 but not in rVI-MC10 cells.

The p-glycoprotein activity was up-regulated only in rVI-MC10 cells. From these findings, continuous exposure of MCT cells to imatinib can induce emergence of a second mutation in ckit that plays an important role in imatinib resistance. Moreover, both KIT-independent activation of ERK and up-regulation of p-glycoprotein activity may also be involved in the development of imatinib resistance in MCT cells when they are exposed to higher concentration of imatinib.

ASSESSMENT OF EQUINE WOUND METABOLISM USING NON- AND MINIMALLY INVASIVE TECHNIQUES

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Hypoxia has been suggested to play a key role in the pathogenesis of exuberant granulation tissue (EGT) and delayed wound healing in horses. Therefore, the purpose of this study was to investigate metabolism and blood flow in the wound bed of experimental equine wounds healing with formation of EGT. Wound healing research in horses is often conducted using repeated collection of biopsies from surgically created wounds, with only one collection from every wound. To obtain sample material less invasively, a microdialysis technique was developed and used to collect wound interstitial fluid from excisional wounds created in six horses. Blood flow in the wound bed was assessed non-invasively by laser Doppler flowmetry.

Measurements were performed repeatedly before and after wounding (until day 28). Both techniques were well tolerated by the horses, and the microdialysis technique provided a simple in composition, small volume sample material (120 uL collected per hour). Blood flow was consistently low in limb wounds with EGT formation, and metabolic analyses showed low glucose concentrations combined with high lactate concentrations in the wound bed of wounds with EGT. In conclusion, microdialysis showed to be an effective method to obtain wound interstitial fluid with minimum discomfort to the horse and allowed for repeated sampling from the same wound. The metabolic disturbances may suggest inadequate oxygen supply during the wound healing process in equine limb wounds healing with EGT. This may be related to the inherently decreased perfusion in the wound bed of limb wounds.

VALIDATION OF A CANINE-SPECIFIC HIGH-SENSITIVITY C-REACTIVE PROTEIN (hsCRP) ASSAY

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C-reactive protein (CRP) is a clinical useful major acute phase protein in dogs, and canine specific methods are available for measuring the marked increases in CRP concentrations observed in dogs with systemic inflammatory disease. However, these tests are often not suitable to detect minor elevations of CRP; instead high-sensitivity CRP (hsCRP) assays are recommended for this purpose. In this study, a previously validated canine-specific CRP assay (Gentian cCRP, Gentian AS, Moss, Norway) was modified with the aim to develop an automated hsCRP assay. A method validation study of the modified assay was performed. Preset quality goals were based on data on biological variation in healthy dogs and the maximal allowable total error (TEa) was set to 29.6%. The measurement range was 0.5-30 mg/l, where 0.5 mg/l was the limit of quantification (LoQ) defined as the lowest concentration where total error (TE) <TEa. The intra- and inter-assay imprecisions, expressed as coefficients of variation, were 2.7% and 3.0% respectively at CRP concentration 2.1 mg/l. The method was linear under dilution; the regression analysis from the linearity study was y = 1.01x-0.3 mg/l. An analytical relevant prozone effect was found for samples with CRP concentrations >150 mg/l. Minor interference from hemolysis was present. Forty-two serum samples were included in a method comparison study with a previously validated canine CRP ELISA (Phase EIA Canine CRP Assay, Tridelta Development Ltd, Maynooth, Ireland); the result from Passing-Bablok regression analysis was hsCRPGentian = 0.93 x CRPELISA -0.31mg/l. In conclusion, the Gentian hsCRP assay had acceptable performance and the method may be used to measure CRP in dogs with sub-clinical inflammatory diseases.

IS LAMP A SOLUTION FOR CHALLENGES AND LIMITATIONS IN DIAGNOSIS OF JOHNE'S DISEASE?

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Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants caused by Mycobacterium avium subsp. paratuberculosis (MAP) is the causative organism of Johne's disease. The lack of rapid and accurate diagnostic test is an important impediment of paratuberculosis diagnosis and control. The objective of the present study was to evaluate the usefulness of LAMP (Loop-Mediated Isothermal Amplification Method) in detecting specific insertion sequence of MAP IS900.

LAMP is a relatively new DNA amplification technique, which due to its simplicity, ruggedness, and low cost could provide major advantages. Our proposed method employs a DNA polymerase and a set of six specially designed primers that recognizes a total of eight distinct sequences on the target DNA.

Our proposed method employs a DNA polymerase and a set of six specially designed primers that recognize a total of eight distinct sequences on the target DNA. 350 faeces and blood samples were collected from dairy cows with 18 months of age and higher from dairy farms of Tehran Province, Iran. Bacterial culture, ELISA and PCR were performed according to the standard protocols. Primers were designed using Primer Explorer V 4 software. 6 specific primers were used including two outer (B3, F3), two inner (BIP, FIP) and two loop primers (LB, LF). Comparison of the results obtained by ELISA, LAMP and PCR showed that there was a significant correlation between LAMP and ELISA results. It was concluded that LAMP is a sensitive and rapid method for detection of MAP.

ANALYSIS OF ACUTE PHASE PROTEINS IN INTERSTITIAL FLUID FROM EQUINE WOUNDS HEALING BY SECOND INTENTION BY THE USE OF MASS SPECTROMETRY

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In horses, the propensity for pathological healing with formation of exuberant granulation tissue (EGT) is a particular problem in limb wounds, and chronic inflammation has been proposed to be a key inducer of EGT. This study aimed to investigate expression of acute phase proteins (APPs) in the wound bed as markers for inflammation. Mass spectrometry (MS)-based proteomics presents a novel approach for absolute quantification of proteins, by the use of selected reaction monitoring (SRM) methods. Experimental wounds were created on 5 horses and were left to heal with formation of EGT or healthy granulation tissue.

Interstitial fluid was recovered by microdialysis from the wounds on days 1, 2, 7 and 14 after wounding. Concentrations of serum amyloid A, fibrinogen, ceruloplasmin, haptoglobin, plasminogen, prothrombin, α-2-macroglobulin, and α-1-antitrypsin were measured with MSSRM.

Concentrations of fibrinogen, haptoglobin, ceruloplasmin, prothrombin, and α-1-antitrypsin were significantly higher in microdialysates obtained from wounds healing with EGT than in microdialysate obtained from wounds healing with healthy granulation tissue, suggesting that there is a state of sustained inflammation in wounds healing with EGT.

This is the first report of absolute quantification of APPs in the horse by use of a MS-based proteomic technique. The technology proved useful for absolute quantification of the investigated proteins and presents a valuable approach for protein analyses in veterinary research and diagnostics in the future.

PROTEOME ANALYSIS OF NASAL SECRETION FROM HEALTHY, MALIGNANT CATARRHAL FEVER (MCF) CHALLENGED AND VACCINATED CATTLE

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Nasal secretion (NS) offers a novel method of quantifying responsiveness to vaccines. In the present study, the aim was to compare the level of alkaline phosphatase (AP) activity in NS from naive and vaccinated, MCF challenged animals and to use Difference Gel Electrophoresis (DiGE) to identify NS proteins differing between groups. Vaccination was at d-0 and boosted at d-30, using an attenuated C500 strain of AlHV-1 with challenge at d-70, using virulent C500 strain of AlHV-1. The AP activity ratio in NS pre (d-56) and post challenge (d-84) was measured and used as the outcome variable (AP response). DiGE separation was carried out and protein spots were analysed with DeCyder 2-D Differential Analysis Software and identified using the SwissProt database. One-way ANOVA was used to select protein spots, which were differentially expressed with P value < 0.05. Primary and booster immunization and challenge via intranasal (IN) or intramuscular (IM) routes had significantly different effects on AP activity in nasal secretion, being highest in the IM/IM treatments (P<0.05).
Median responses (ratio of AP at d-84:d-56) were 0.69, 0.72, 0.77 and 2.4 for PBS, Adjuvant, IM/IN, IM/IM treatments respectively. Following trypsin digest and peptide mass fingerprinting 23 proteins were significantly differentially expressed as a result of IN challenge and IM vaccination. AP response to vaccination and challenge differed significantly with treatment, being strongest after two IM treatments, suggesting an adaptive immune component in the regulation of nasal AP secretion. Quantitative proteomics technology such as DiGE has identified and measured changes in nasal protein expression in response to MCF and following vaccine protection.

Poster presentations

EFFECT OF HIGH FAT DIET ON PERIPHERAL BLOOD LEUKOCYTE TRANSCRIPTOME OF RELEVANT ENERGY HOMOEOSTASIS GENES ON CATS

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Peripheral blood leukocyte (PBL) transcriptomes have been used for investigating the molecular mechanisms underlying several human diseases. As such, the aim of this preliminary study was to determine the sensitivity of cat PBL to a high fat diet (HFD), by assessing for changes occurring in PBL mRNA transcriptome profiles of genes mainly involved with energy homoeostasis (MDH, G6PDH, FAS), metabolic homeostasis (AMPK), insulin signaling (IRS-1, IRS-2, PI3K), adiponectin signaling (ADIPOR1, ADIPOR2). Plasma metabolite profiling highlighted early signs of liver distress (significant increase in ALT, AST, and ALP) due to higher dietary fat intake. Moreover, PBL transcriptome trends revealed early signs of high-fat obesity linked inflammation (reduced ADIPOR1, ADIPOR2, and AMPK expression) and possible fatty acid induced insulin resistance (reduced IRS-1 and IRS-2). In addition, with increased dietary fatty acids coming from the HFD, metabolism of exogenous fatty acids appeared to increase (greater MDH expression), whereas fatty acid synthesis was reduced (reduced G6PDH and FAS expression), since tissues need not synthesize fatty acids, absorbing them from circulation. In summary, our results indicate that PBL appear to be sufficiently sensitive to HFD induced alterations to transcriptomes of genes to offer us a snapshot of the overall state of metabolic changes occurring in the body, especially with certain genes associated with obesity and diabetes risk. Further work and validation is required to determine whether PBL transcriptome trends can be extended to distal tissue.

APPLICATION OF ACUTE PHASE PROTEIN ASSAYS IN AVIAN, EXOTIC, AND WILDLIFE SPECIES

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Acute phase protein (APP) assays have primarily been described for use in companion animals and large animals and quantitation of APP has been demonstrated to be very sensitive for the detection of inflammation and infection in this species. Given the conservation of these proteins, it has been reasonable to assume that APP reagents will have the potential to cross react with other species. An initial published investigation was undertaken a few years ago and supported this premise. In recent years, our laboratory has sought to validate commercially available serum amyloid A (SAA) and C-reactive protein (CRP) reagents in several avian, exotic, and wildlife species. Results have been mixed. Positive reactivity for anti-human CRP reagents has been observed in rabbits. Anti-human SAA reagents have been found to be cross reactive with elephant, manatee, and zebra species. These same reagents have recently been reported to detect SAA in falcons by others although we have failed to demonstrate reactivity in penguins. This presentation will reiterate the tenets of validation and show the utility of APP quantitation in some wildlife species. This will include some recently published data as well as new data acquired from active projects underway as of this writing.

MULTIPLE IMMUNOFLUORESCENCE STAINING FOR THE CLASSIFICATION OF LYMPHOCYTE IMMUNOPHENOTYPES IN CANINE AND FELINE LYMPHOMA

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Classification of the lymphocyte immunophenotype in canine and feline lymphoma, such as B- or T-cell lymphoma, is important for the prognosis and development of treatment protocols. For diagnostic cytology, some more practical tests that use smear samples are required for immunophenotyping of advanced lymphomas. The aim of this study was to develop a multiple immunofluorescence staining method for the detection of lymphocyte immunophenotypes in cytological specimens and to evaluate its clinical utility. B- and T-cells were detected using anti-CD79α and anti-CD3 antibodies, respectively, followed by specific fluorescence-labeled secondary antibodies. The multiple immunofluorescence staining method was first developed using frozen sections of normal lymph nodes. The optimal concentration of fixative, necessity of antigen retrieval, and concentration of the antibodies were determined. The method was then applied to impression smears of normal lymph nodes and clinical samples from dogs and cats with lymphoma. B- and T-cells were detected with specific signals in the frozen sections using formalin fixation without antigen retrieval. Specific signals were also detected in the impression smears of the normal lymph nodes and lymphoma tissue samples, and the results of this immunofluorescence staining method corresponded with those of a genetic clonality analysis. The multiple immunofluorescence staining method that we developed in this study effectively distinguished the lymphocyte immunophenotypes with high specificity and sensitivity using only 1 smear sample, and was useful as a diagnostic tool for canine and feline lymphoma.

INFECTIOUS AGENTS AND CO-INFECTIONS IN ANEMIC AND NONANEMIC CATS

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Infectious diseases in anemic cats are mainly caused by retrovirus, hemoparasites, or its association. Identification of co-infections is important to evaluate severity of an illness, prognosis and adequate monitoring. The objective of this study was to determine the prevalence of infection and co-infection by retrovirus and hemoparasites in anemic and non-anemic domestic cats and evaluate the severity of disease. Blood samples from 142 domestic cats, 40 anemic (group I), 50 non-anemic (group II) and 52 healthy cats (group III) were evaluated. Real time PCR was performed for the followings agents: Anaplasma spp., Bartonella spp., Cytauxzoon felis, Ehrlichia spp., Mycoplasma haemofelis, 'Candidatus Mycoplasma haemominutum', 'Candidatus Mycoplasma turicensis', feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV); also ELISA method was performed for FIV and FeLV. Of the total samples, 41.5% (59/142) were positive for at least one agent, with 64.4% (38/59) presenting single infection and 35.6% (21/59) co-infections. Co-infection between retroviruses and hemoparasite was more prevalent in group I. The most prevalent agent in anemic cats was FeLV, present in 57.5% (23/40) of samples, and more than half of anemic cats infected only by this agent showed nonregenerative anemia. Co-infection between retroviruses and hemoparasite increases the severity of the disease. Anemic cats infected or co-infected by hemoparasites and retroviruses are more likely to die than non-anemic cats. This study showed a high prevalence of co-infections in anemic cats and the existence of asymptomatic carriers co-infected. Identification of these agents and their associations should be performed to establish adequate monitoring and prognosis

VALIDATION OF TWO IMMUNOTURBIDIMETRIC METHODS FOR THE DETERMINATION OF THE ACUTE PHASE PROTEINS PIG-MAP AND CRP IN PIG SERUM SAMPLES

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The serum concentration of acute phase proteins (APP) increases in the presence of disease or stress, which makes APP notable parameters for the global assessment of animal health and welfare. APP measurements in animal production have demonstrated to be useful for the evaluation of herd health status, in the detection of subclinical infection or stress conditions causing poor productive performance, as well as for ante/post mortem inspection, or in studies aimed to evaluate the efficiency of antibiotic or antiinflammatory treatments or vaccination. Pig Major acute phase protein (pig-MAP) and C-reactive protein are two of the main APP in pigs. The concentration of APPs in pig serum are usually measured by ELISA, a technique with a relatively high variability. Two robust, immunoturbidimetric methods for the determination of Pig-MAP and CRP (particle enhanced method) have been developed using specific antibodies raised in rabbits. The methods developed show good precision (Intra assay CV < 5%; Inter-assay CV < 10%) and accuracy, with a linearity range up to 5 mg/mL for pig-MAP and 0.1mg/mL for CRP. A good correlation was observed between the values obtained with the turbidimetric methods and alternative methods such as ELISA or radial immunodifussion (R>0.98). No interference due to the presence of hemolysis (20g/L hemoglobin), lipaemia (4-10g/L triglycerides) or bilirrubin was detected.
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HEMATOLOGICAL VALUES, TOTAL PLASMA PROTEIN AND HETEROPHIL: LYMPHOCYTE RATIO OF BLACK-FRONTED PIPING GUAN (ABURRIA JACUTINGA) KEPT IN CAPTIVITY

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The hematological evaluation in birds has not been documented in many species and provides information about diseases, rehabilitation and health of populations and its monitoring. The heterophil:lymphocyte (H/L) ratio is a parameter useful for detecting stress. The black-fronted piping guan (Aburria jacutinga) is a Cracid considered threatened in Brazil. This study aimed to contribute to hematological, total plasma proteins (TPP) and H/L ratio values of guans kept in captivity. Blood samples from 32 guans were collected for the complete blood count and determination of TPP and from eight young animals for leukocyte differentiation. Results for adult animals were: erythrocytes (x106/µL): 2.08 ± 0.34; PCV (%): 43 ± 04; hemoglobin (g/dL): 10.6 ± 1.1; MCV (fL): 209.4 ± 25.1 and MCHC (g/dL): 24.8 ± 1.5; total leukocytes (/µL): 12,968 ± 7,605; heterophils (%): 37 ± 11; lymphocytes (%): 44 ± 12; eosinophils (%): 6 ± 3; monocytes (%) 8 ± 4; basophils (%): 5 ± 3 and H/L ratio: 1.0 ± 0.6. Thrombocytes were normal. The value of TPP was 5.3 ± 2.0 g/dL. Leukocyte differential for the young animals were: heterophils (32%); lymphocytes (42%); eosinophils (2%); monocytes (16%); basophils (7%); H/L ratio: 0.76. Hematologic values may vary according to geographic location, habitat, nutrition, age, stress and health. Knowledge laboratory parameters for guans (Aburria jacutinga) are of importance in the conservation of this species and in breeding endangered guans species in captivity for later release.
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HAPTOGLOBIN IN FEMALE DOGS SUBMITTED TO CONVENTIONAL AND MINIMAL INVASIVE OVARIO-HYSTERECTOMY

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Thirty healthy adult female dogs were submitted to ovario-hysterectomy by two different techniques: minimal invasive, with the use of nylon cables ties (G1), and conventional, with the use of nylon sutures (G2) for the ligature of ovarian pedicles and uterine body. Serum samples were obtained in four different time points: before surgery and at 24 hours, 48 hours and 7 days after the surgical procedure. Serum haptoglobin concentrations were measured via haemoglobin binding assay previously validated for use in dogs. The Kruskal-Wallis one way analysis of variance by ranks was performed to investigate the intragroup median, and the intergroup median was analyzed with Mann-Whitney U test. All tests were performed at the ($P \leq 0.05$) significance level. There was no significant difference ($P \geq 0.05$) between the time points in the G2. In the G1, the haptoglobin concentrations was significantly increased ($P \leq 0.05$) 24 and 48 hours after surgery (4.5g/L and 4.9g/L respectively). On day 7, these concentrations returned to the same values found before surgery (2.2g/L and 0.98g/L respectively). It is known that increased haptoglobin concentrations are not only indicative of inflammation, but this APP is sensitive to the effects of corticosteroids that might result from cortisol release due to stress. Thus, suggesting that the higher concentration of haptoglobin found 24 and 48 hours after surgery was caused not only by inflammation caused by the surgical trauma, but also due to stress in the early postoperative period caused by the use of the nylon cable ties.

HEMATOLOGICAL AND TOTAL PLASMA PROTEIN VALUES OF FREE-LIVING RED-TAILED AMAZON PARROT (Amazona brasiliensis) NESTLINGS

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The red-tailed Amazon parrot (Amazona brasiliensis) is an endangered species of psittacine and endemic in South and Southeast Brazilian Atlantic coastal region. Hematological and total plasma protein profiles were determined for 33 free-living nestlings' parrots in Paraná state, Brazil. Parrots were taken from the nest and manually restrained for body weight, physical examination and blood collection. Thirteen birds had an average weight of < 400 g (G1) and 20 birds had an average weight of > 400 g (G2). The mean values and the standard deviation values for G1 were: erythrocytes – 1.30 x 106/µL (±0.58); packed cell volume – 31.25% (±5.34); hemoglobin – 10.63 g/dL (±2.21); white blood cells count – 47.33 x 10³/µL (±16.25); mature heterophils – 72.5% (±15.1); immature heterophils – 0% (±0); lymphocytes – 20.9% (±12.2); eosinophils – 2.6% (±4.6); monocytes – 1.6% (±2.2); basophils – 2.4% (±2.4); TPP – 2.6 g/dL (±0.4). The values for G2 were: erythrocytes – 1.79 x 106/µL (±0.94); packed cell volume – 32.95% (±6.78); hemoglobin – 9.26 g/dL (±2.74); white blood cells count – 33.05 x 10³/µL (±18.29); mature heterophils – 71.8% (±13); immature heterophils – 1.2% (±3.5); lymphocytes – 23.9% (±11.7); eosinophils – 2.2% (±3.1); monocytes – 0.2% (±0.7); basophils – 0.3% (±0.6); TPP – 2.91 g/dL (±0.72). This is the first study documenting hematological and protein values for the Amazona brasiliensis, since it is a free-living population. Parameters obtained in this study contribute to a hematological database for wildlife animals, especially for threatened species.

NEW MONOCLONAL ANTIBODIES SPECIFIC TO CANINE NT-PROBNP ALLOW DEVELOPMENT OF SENSITIVE IMMUNOASSAYS WITH HIGH DYNAMIC RANGE AND IMPROVED APPARENT STABILITY OF THE ANALYTE

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NT-proBNP is a useful marker of heart failure in dogs. However, a new generation of canine NT-proBNP immunoassays is needed in order to overcome the problems of first-generation immunoassays such as narrow dynamic range and low apparent stability of the analyte in vitro. The aim of this study was to obtain a panel of monoclonal antibodies (mAbs) specific to canine NT-proBNP and use them for development of prototype immunoassays avoiding these shortcomings.

Each of the 65 newly developed mAbs specific to different regions of canine NT-proBNP was tested as a capture and detection antibody (labelled with stable europium chelate) in sandwich immunoassays with endogenous and recombinant NT-proBNP.

Five immunoassays demonstrated very high sensitivity for recombinant NT-proBNP (25 pg/ml) and were selected for analyzing plasma samples of dogs with heart disease. Significantly higher NT-proBNP concentrations were observed in the group of dogs with heart disease comparing with group of healthy dogs. No plasma dilution step was required even for samples with high NT-proBNP level due to the wide dynamic range of the developed assays.

In addition, NT-proBNP remained stable in EDTA plasma samples for at least 72 hours at +4°C.

In conclusion, these new mAbs can be used to develop a sensitive canine NT-proBNP immunoassay with an improved performance as a result of increased apparent stability of the analyte and minimized need for sample dilution.

TEMPORAL CHANGES OF SERUM AMYLOID A AND HAPTOGLOBIN CONCENTRATIONS IN NEWBORN LAMBS AND ASSOCIATION TO THE WEIGHT GAIN

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The aim of the study was to investigate changes in serum amyloid A (SAA) and haptoglobin (Hp) serum concentrations in postnatal lambs and study their associations with lambs' weight gain at 3-4 months of age. Serum samples from 322 lambs were obtained at 0-25 days of age (1-3 samples from lamb, n = 524). Lamb's weight was recorded 3-4 months after birth. SAA concentrations were measured with ELISA, and Hp with the hemoglobin binding assay. Linear random-intercept models were used for statistical analysis. For evaluating factors associated with weight gain lambs gender, SAA or Hp concentrations from different weeks, sibling's number, age at sampling, and sampling year (2011 or 2012) were fixed factors and ewe as random factor. SAA concentrations were low after birth at day 0 and highest at first day.
Concentrations stayed elevated until 5 days, decreased at day 6 and then stayed stable. Higher SAA concentrations at 6-12 days of age (n = 194; mean 16.3 +/- 32.9 mg/l) were associated ($p < 0.001$) with lower weight gain (244.5 +/- 55.1 g/day). Hp concentrations were lowest at first week of life starting to increase after that but at average Hp concentrations were significantly higher at year 2012 ($p < 0.001$). Higher Hp concentrations at 6-12 days of age (n = 105; 188.4 +/- 230 mg/l) were associated with lower weight gain in year 2012 but not in year 2011 (n = 89; 144.9 +/- 306.9 mg/l). Associations of higher SAA and Hp during second week of life with lower weight gain suggest that infections at that time influence lambs' development throughout the first months of life.

POIKILOCYTOSIS IN RABBITS: PREVALENCE, TYPE, AND ASSOCIATION WITH DISEASE

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Rabbits (Oryctolagus cuniculus) are a popular companion animal and animal model of human disease. Abnormal red cell shapes (poikilocytes) have been observed in rabbits, but their significance is unknown. The objective of this study was to investigate the prevalence and type of poikilocytosis in rabbits and its association with physiologic factors, clinical disease, and laboratory abnormalities. We retrospectively analyzed 692 blood smears from 503 rabbits presented to the University of California Veterinary Medical Teaching Hospital from 1990 to 2010. Number and type of poikilocytes per 2000 red cells were counted and expressed as a percentage. Acanthocytes were observed in 583/692 (84%) samples and comprised >3% (maximum 72.2%) of red cells in 228/692 (33%) samples. Echinocytes were observed in 383/692 (55%) samples and comprised >3% (maximum of 71.1%) of red cells in 161/692 (23%) samples. Significant differences were not observed with sex, age, or breed, but native rabbits (n=7) had the smallest range of poikilocytes (0.3–1.9%). Fragmented red cells (keratocytes, schistocytes, microcytes, spherocytes) were found in 253/692 (36.5%) samples, comprised >0.5% (maximum 4.7%) of red cells in 38/692 (5.6%) samples, and were more prevalent in samples from diseased (n=599) than healthy (n=93) rabbits (P=0.042).
Fragmentation (together with acanthocytosis) was significantly associated with abscesses, inflammatory disease, anemia, polychromasia, heterophilia, lymphopenia, thrombocytosis, hypoalbuminemia, hyperglobulinemia, hyperfibrinogenemia, and hypercholesterolemia ($P<0.05$). Echinocytosis was significantly associated with renal disease, azotemia, and acidbase/electrolyte abnormalities ($P<0.05$). These findings provide important insights into underlying pathophysiologic mechanisms that appear to affect the prevalence and type of poikilocytosis in rabbits. Our findings also support the need to carefully document poikilocytes in clinical diagnosis and in research investigations and to determine cut-offs for assessing diagnostic and prognostic value.

SIRTUIN 1 SUPPRESSES INFLAMMATION BY p65/RelA PATHWAY AND p65/RelA REDUCES THE EXPRESSION OF SIRTUIN 1 mRNA LEVELS IN CAT CULTURED CELLS

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Sirtuin 1 (SIRT1) is a member of Sir2 like NAD-dependent deacetylase, and it plays important roles in maintaining metabolic functions and immune responses. We performed that high fat diet feeding studies for 6 weeks in cats, the hepatocellular inflammatory markers (ALT, AST, and ALP) in peripheral blood are increased and the expression of SIRT1 mRNA levels is decreased in liver tissue. But the detail of molecular mechanism of inflammation and SIRT1 is still unknown. NF-κB plays a key role in regulating inflammation, in particular p65/RelA subunit has the ability to regulate immune-related gene expressions independently. In this study, we investigated the interaction of SIRT1 with p65/RelA in cats. We obtained cat SIRT1 and p65/RelA cDNAs, and cloned into expression vector. To determine the interaction of cat SIRT1 and p65/RelA, we transfected the prepared vectors to cat fibroblast cells and performed luciferase reporter assay and realtime PCR. Luciferase assay revealed that SIRT1 suppressed the NF-κB reporter activity by p65/RelA pathway. Realtime PCR revealed that p65/RelA overexpression elevated the expression of pro-inflammatory cytokine (IL-1, IL-6, TNF-α) mRNA levels and reduced the expression of SIRT1 mRNA levels in cultured cells.
These results suggest that SIRT1 and p65/RelA interact each other and regulate inflammation.

CLINICAL EVALUATION OF A POINT-OF-CARE IN VITRO DIAGNOSTIC SYSTEM FOR MEASUREMENT OF SERUM CRP IN DOGS EXPERIMENTALLY INFECTED BY LEISHMANIA INFANTUM

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C-reactive protein (CRP) is an acute phase protein and an important component of the innate immune system that may be of interest in the diagnosis and monitoring of canine leishmaniasis, an endemic disease in the Mediterranean ecoregions. LifeAssays® (Lund, Sweden) VetReader is a veterinary point-of-care in vitro diagnostic system that provides routine measurement of canine CRP. The objective of this study was to perform a biological validation study of this test using Leishmania infantum experimentally infected dogs. Ten 18 month old Beagle dogs were IV infected with 5 × 107 promastigotes of L. infantum and 3 dogs were included as control. Dogs were monitored for 15 months by means of hemogram, clinical chemistry, parasite load (qPCR), L. infantum-specific serology and LifeAssays Canine CRP diagnostic system A clinicopathological scoring system (CPS) which takes into account clinical signs and laboratory parameters was build to follow the progression of the disease. CPS was altered at 323 days post-infection, indicating the presence of clinical symptoms, which were mild throughout the experiment. Parasite load was detected in serum at 240 days. Humoral immune response presented significant increases at the end of the study. Experimental infection induced an increased in CRP which was statistically significant about 10 months after infection (levels > 20 mg/L). In conclusion, the present study indicates that the new point-of-care is suitable for monitoring experimental canine leishmaniosis.

COMPARISON OF TOTAL ALLOWABLE AND TOTAL OBSERVED ERROR FOR 2 CHEMISTRY ANALYZERS

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Total observed error (TEobs) from two chemistry analyzers in two laboratories with well-established quality management systems were compared with recommendations for total allowable error (TEa) recently published by the ASVCP; sigma values were also calculated. Internal quality control (QC) data for a 3 month period was reviewed. Both laboratories ran 2 levels of QC material once daily.

The first analyzer was a Siemens Dimension ExL (DIM) in a commercial laboratory. The QC strategy consisted of using the 3s rule and monitoring TEobs versus TEa. TEobs was less than TEa for all analytes except bile acids (TEa 20% TEobs 42%) and glutamate dehydrogenase (TEa 25% TEobs 72%): both of these were run as open channels with reagents from a different manufacturer. Calculation of the quality goal index (QGI) revealed that failure to meet the TEa was due to imprecision in both cases, which exceeded the manufacturer's claims. The second analyzer was a Roche Cobas Integra c501 (COB) in a university laboratory. The QC strategy consisted of using the 2s rule. TEobs was slightly higher than TEa for sodium, potassium and chloride (TEa all 5%, TEobs all 5.5%). QGI calculation revealed that excess imprecision (exceeding the manufacturer's claims) was the problem. When examining sigma metrics, 13/25 analytes on the DIM and 16/25 analytes on the COB achieved a sigma value of >6.0. Analytes with sigma <3.0 were those not meeting the TEa goals. In conclusion, ASVCP TEa goals were achievable for most analytes and indicated a sigma metric ≥3.0. Different analytes failed on the two analyzers; control material preparation, stability of reagents and mechanical causes should be investigated.

CHANGES IN PLASMA LIPOPROTEIN PROFILES AND MALONDIALDEHYDE CONCENTRATIONS IN HYPERLIPIDEMIA DOGS

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Background: The aim of this study is to compare metabolic parameters, malondialdehyde as a lipid oxidation marker, and lipid profiles between dogs with hyperlipidemia with and without treatment, in order to examine the usefulness of malondialdehyde and lipid profiles as diagnostic parameters at early stages of hyperlipidemia. Results: Dog samples were collected from four different veterinary clinics across Japan from March to June 2013. They were separated into three groups: control, untreated hyperlipidemia based on temporally screening, and hyperlipidemia with current anti-hyperlipidemic (statins and fibrates) treatment. Plasma triglyceride levels of untreated hyperlipidemia dogs were significantly higher than those of control dogs. ALT levels of hyperlipidemic dogs with treatment were the highest among three groups. VLDL and LDL of both cholesterol and triglyceride of untreated hyperlipidemia dogs were the highest among three groups. HDL1 levels in triglyceride of hyperlipidemia dogs with treatment were significantly higher than those of control and untreated hyperlipidemia dog. Malondialdehyde concentrations of untreated hyperlipidemia dogs were significantly higher than those of control and hyperlipidemic dogs with treatment.
Conclusions: In this study, dogs with untreated hyperlipidemia clearly showed abnormal status in lipid metabolism, whereas hyperlipidemic dogs under anti-hyperlipidemia treatment showed more normal lipid status suggesting the effectiveness of the therapy. Antihyperlipidemics (statins and fibrates) for dogs are also effective in relieving elevated levels of lipids and lipid oxidation. Plasma lipid (triglyceride and cholesterol) profiles and malondialdehyde are useful diagnostic tools for identifying early stages of untreatment hyperlipidemia in dogs.

REFERENCE INTERVALS FOR BRONCHOALVEOLAR LAVAGE CYTOLOGY IN STABLED CLINICALLY HEALTHY HORSES

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Bronchoalveolar lavage (BAL) entails the sampling of pulmonary epithelial lining fluid (PELF) from the terminal bronchioles and alveoli. However, still more than thirty years after its introduction, there is no international standardized method for performing BAL, including agreed reference intervals (RIs) based on scientific studies. BAL is an invaluable tool for equine lower airway diagnostics, whereby better scientific justified RIs will improve the diagnostic value of the test results. The objective of this study was to establish RIs for total and differential cell counts in equine BAL samples. Seventy-eight stabled clinically healthy horses, aged 5-27 years and twelve horses diagnosed with recurrent airway obstruction (RAO), aged 6-19 years were included in the study. Blood and BAL samples were collected and BAL cytology evaluated by several observers. The RIs were calculated using the nonparametric 95% confidence interval method. BAL cytology RIs includes: alveolar macrophages [47.0; 88.0%], lymphocytes [6.5; 44.5%], neutrophils [<15.0%], eosinophils [<1.5%] and mast cells [<6%]. RI for total cell count were [<0.9x109 cells/L]. The RIs for BAL fluid cytology estimated in this study are the first prospectively constructed RIs for stabled clinically healthy horses and valid for stabled horses between 5-27 years. Both the BAL procedure and subsequent laboratory methods for cytological evaluation used in this study are easy to adopt and thus relevant for equine field practice conditions.

RELATIONSHIP BETWEEN MILK AMYLOID A, SELECTED PROTEINS, AND ELECTROPHORETIC PATTERN OF BOVINE SERUM MILK PROTEINS USING SDS-PAGE IN SUBCLINICAL MASTITIS CAUSED BY COMMON PATHOGENS IN IRAN

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For detection the invisible changes in subclinical mastitis and identification of the constituent proteins of infected animals new biomarkers and methods are used. The aim of this study was to determine milk protein changes in subclinical mastitis, considering their electrophoretic pattern. 59 clinically healthy cows were randomly selected. Of these, 41 cows were considered to have subclinical mastitis based on a Somatic Cell Count (SCC) higher than 100×1000 cells/mL of milk and positive bacterial culture. These samples were analyzed for Total protein, Milk Amyloid A (MAA) and Immunoglobulins using a commercial ELISA kit. The electrophoretic patterns of samples were studied with the SDSPAGE technique. Significant ($P<0.001$) differences were seen in the mean concentration of SCC, Total protein and MAA, Immunoglobulins between the healthy cows and subclinical mastitic cows with. The electrophoretogram showed that higher molecular weight bands appeared in the milk of mastitic cows in the range of 70-170KDa. In this range, the mastitic samples had 2-5 bands.
Staphylococcus aureus was the most common pathogen, with 12 (29.26%). In conclusion MAA concentrations below the detection limit were considered as good indicators of healthy udder quarters. On the basis of the different results between the electrophoretic patterns of milk from healthy and mastitic cows, SDS-PAGE is a suitable method for the diagnosis of cows with subclinical mastitis.

COMPARISON OF PLASMA CHOLESTEROL AND TRIGLYCERIDE PROFILES AND METABOLITE CONCENTRATIONS BETWEEN AGED DOGS AND YOUNG DOGS

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In dogs, incidence of lipid metabolism disorders such as obesity and diabetes mellitus has increased markedly. Hyperlipidemia has been regarded as a common sign of obesity and hyperlipidemic condition may be associated with inflammation, oxidative stress and lipid composition changes. In this study, we investigated the changes in plasma cholesterol and triglyceride [TG] profiles and metabolite concentrations in 24 dogs (young: 0-7 years old, n=12, aged: 8-13 years old, n=12). Mean plasma adiponectin [ADN] concentration was significantly lower in aged dog group than than in young dog group. Although there was no significant difference statistically, aged dogs had higher plasma alpha1- acid glycoprotein (alpah1-AG) level compared to that in young dogs. Plasma cholesterol and TG lipoproteins were divided into four fractions by biphasic agarose gel electrophoresis technique. The level of the third TG-lipoprotein fraction from the positive pole (TG Fraction 3) was significantly higher in aged dogs than in young dogs. On the correlation coefficient analysis by Peason's method, moderate positive correlations were seen between the age, and TG ($r = 0.446$, $P = 0.029$), TG Fraction 3 ($r= 0.516$, $P = 0.010$), malondialdehyde ($r = 0.146$, $P = 0.043$), alpha-1 AG ($r = 0.448$,$P = 0.028$) levels, respectively. Moderate negative correlations were seen betweein the age, and total cholesterol (TC) Fraction 2 ($r = -0.446$, $P = 0.029$), glucose ($r = -0.637$, $P = 0.001$), ADN ($r = -0.408$, $P = 0.048$), respectively. Present data suggest characteristics of lipid metabolism disorder may be affected by aging in dogs.

SERUM IRON, FIBRINOGEN AND LEUKOCYTE COUNT AS INFLAMATORY MARKERS IN HORSES

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Determining the degree of inflammation in sick horses is crucial for therapeutic procedures as well as to ascertain the prognosis of the disease. Blood inflammatory markers, such as fibrinogen, total white cell count and serum iron can be used to identify and monitor the inflammatory response. However, iron determination is rarely made during clinical routine of horses and there is little information on the association between serum iron levels and classical markers of inflammation. The study aimed to compare the levels of serum iron with fibrinogen and total white cell count in horses with acute inflammation of different etiologies, examined at the Veterinary Hospital of UFPR. A total of 47 animals with gastrointestinal and respiratory disorders, fractures, infections and injuries in skin and appendages were analyzed. For comparison purposes, the first laboratory examination was used. In order to set the reference range of the studied parameters, blood samples from 30 healthy horses were collected. The mean concentration of iron found in the group of patients horses was lower (84,8µg/dL) than the reference interval minimum (91,0µg/dL). The results showed that the change in iron concentration was related to inflammatory condition, when compared to change in both fibrinogen ($p = 0.016$) and total white cells ($p=0.014$). There was no significant difference between fibrinogen and total leukocyte count ($p=1$). Based on the results observed in this research, it is possible to conclude that serum iron may be used as an inflammatory marker in horses, which is more reliable in cases of acute evolution.

ANALYSIS OF PEPIDES AND PROTEINS IN SWINE SALIVA WITH MALDI-TOF IS NOT ALWAYS SUCCESSFUL

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Swine saliva, also known as oral fluids, can be easily obtained with cotton ropes. Many different tests are possible in collected fluids. The use of oral fluid specimens in diagnostics and surveillance provides many advantages over serum as it is a non-invasive sampling technique. However, there are also disadvantages. The goal of our study is to detect peptides and small proteins (< 20.000 Da) that gives an indication of the immune function of the animals. We used MALDI-TOF (Matrix Assisted Laser Desorption Ionization – Time of Flight Mass spectrometry) to detect the peptides and proteins. Ropes were used according to Jeff Zimmerman's instructions: Cotton ropes were hanged in swine stables for 30 minutes, then saliva was collected and send to our laboratory. It was impossible to use ropes in a clean environment without feed and feces, as required. Peptides and proteins were detected with MALDI-TOF MS after treatment of the samples with matrix (HCCA: alfa-cyano-4-hydroxycinnamic acid). Effect of storage of saliva samples at different temperatures was evaluated. However, some samples gives no signal in the MALDI TOF MS. Treatment and different cleaning techniques were tried, but still we could not detect peptides and proteins in these samples. It seemed that contamination with feces in these samples was the main reason for bad results. In other samples, we could detect about 10 to 15 different peptides in the mass range from 900 to 1500 Da and about 10 – 40 proteins in the mass range from 1500 to 25000 Da. Although MALDI TOF MS is a promising technique for detecting peptides and proteins in swine saliva, we have to solve the problem of contamination.

TRIAL OF INSULIN PRODUCING CELLS GENERATION

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Insulin is a critical hormone in the regulation of blood glucose levels and is produced exclusively by pancreatic islet beta-cells. Insulin deficiency due to reduced pancreatic islet beta-cell number underlies the progression of diabetes mellitus, prompting efforts to develop beta-cell replacement therapies. However, precise information on beta-cell replacement and differentiation in canines is limited. In this study, we established insulin-producing cells from bone marrow derived mesenchymal stem cells transiently expressing canine pancreatic and duodenal homeobox 1 (Pdx1), beta cell transactivator 2 (Beta2) and V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A (Mafa) using a gene transfer technique. Real-time PCR analysis revealed an increase in insulin mRNA expression of transfected cells. And ELISA revealed that insulin protein expressed was detected in cytoplasmic fraction. Insulin immunostaining analysis was performed and observed in cytoplasmic fraction. These results suggest that cotransfection of Pdx1, Beta2 and Mafa induce insulin production in canine BMSCs. Our findings provide a clue to basic research into the mechanisms underlying insulin production in the canines.

cDNA CLONING AND mRNA EXPRESSION OF CAT GPR40

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GPR40 is a member of the G-protein coupled receptor (GPCR) family and it is also identifed as long-chain free fatty acid (FFA) receptor. Long-chain FFA amplify glucose-stimulated insulin secretion from pancreatic beta cells by activating GPR40. In this study, we performed cDNA cloning for cat GPR40 and characterized expression profiles of its mRNA in cat tissues. Total RNA was extracted from cat tissues and GPR40 cDNA was cloned by RT-PCR using primers designed from the putative GPR40 gene sequence appeared in cat genome database. The cDNAs for 3'- and 5'- regions were cloned by RACE-PCR. The cloned full-length GPR40 cDNA was bout 1.6 kb. A deduced 320 amino acid of cat GPR40 displayed high overall sequence identity with GPR40 of dog (94%), human (87%) and chimpanzee (86%). Realtime PCR analysis showed that cat GPR40 mRNA was expressed in adipose tissue, bone marrow, duodenum, pancreas, skin, spinal cord, spleen, stomach and markedly high levels in pancreas. These results suggest that GPR40 gene is conserved and may be involved in insulin-secretion from cat pancrease.

BIOMARKERS OF HAEMOSTASIS, ENDOTHELIAL DISFUNCTION AND INFLAMMATION IN BABESIOSIS OF DOGS CAUSED BY BABESIA CANIS CANIS

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Canine babesiosis is a tick-borne disease caused by the haemoprotozoan parasites of the genus Babesia. The aim of this study was to determine changes in coagulation and fibrinolytic parameters, and their association with proinflammatory mediators and endothelium activation. In this study 30 dogs naturally infected with Babesia canis canis and 10 healthy dogs were included. Blood samples from dogs with babesiosis were taken at the day of admission before treatment, and on the 6th day after treatment. Levels of thrombomodulin (TM), high mobility group box-1 protein (HMGB-1), soluble intercellular adhesive molecule-1 (ICAM-1), and soluble urokinase receptor of plasminogen activator (suPAR) were determined using a specific canine ELISA. Level of antithrombin III (AT III) was determined using chromogenic substrate test. Concentrations of TM, HMGB-1 and suPAR were increased in dogs with babesiosis at admission day comparing with healthy dogs, while AT III level was decreased. On the 6th day of disease, levels of AT III and TM were decreased comparing with healthy dogs. Comparing first and 6th day of disease, we found increased levels of TM, ICAM-1 and HMGB-1, and decreased level of AT III on first day of disease. Inflammation caused by parasite and host response to parasite leads to activation of haemostatic system. Results of this study clarify bimodal interplay between coagulation and inflammation, leading to different clinical presentations in babesiosis and presents novel potential biomarkers in veterinary medicine.

HAEMATOLOGICAL VALUES IN HEALTHY PIGS FROM SMALL FARROW-TO-FINISH FARM

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The study was conducted on one-site farrow-to-finish pig farm with 50 sows and 1 boar, free of classical swine fever and Aujeszky's disease. Herd was vaccinated against Mycoplasma hyopneumoniae and atrophic rhinitis. Pigs were dewormed regularly. On the farm they weaned 21.20 pigs per sow per year. The aim of the study was to investigate haematological values in pigs bred in farrow-to-finish farm.

In total, 39 sows, 10 young sows and 6 weaners 10-12 weeks old were sampled. In blood samples haematological variables; red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), haematocrit (Ht), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), number of platelets (PLT) and white blood cell count (WBC) were measured. In blood smears differential leukocyte count was performed. Statistical analysis was performed with SPSS (Ver. 20) software.

Mean values of haematological variables in sows were RBC 5.76±0.58 x1012/L, Hb 12.18±1.14 g/dl, Ht 36.60±3.74 %, MCV 63.61±3.16 fL, MCH 21.18±1.17 pg, MCHC 33.32±0.77 g/dl and WBC 16.29±3.32 x109/L. Median value of PLT was 210.00 109/L. In the young sows and weaners significantly higher number of RBC and WBC and significantly lower value of MCV, MCH and MCHC was established than in adult sows.

Our results are in accordance with published data and indicate, as we expected, that the age of pigs must be taken into account for appropriate interpretation of the haematological data. Further studies on larger numbers of samples are required to obtain useful reference values for our breeding conditions.

HEMATOLOGIC AND SERUM BIOCHEMICAL CHANGES IN DOGS NATURALLY INFECTED WITH DIOCTOPHYME RENALE

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Dioctophyme renale is a large nematode parasite. It is usually found in the renal parenchyma and free in the abdominal cavity. For this study, a total of 14 mixed breed dogs were evaluated. All animals had D. renale ova in the urine sediment and were submitted to nephrectomy as treatment. Blood samples were obtained before surgery and 28 days after the surgical procedure. Haptoglobin, serum amyloid A, C-reactive protein, albumin and total protein concentrations were determined in serum samples. The complete hemogram was also performed. Haptoglobin, total protein values and the number of monocytes were significantly higher ($P<0.05$) before surgery (during infection) and the albumin (as a negative APP) was significantly lower ($P<0.05$) at the same time point and total protein concentration was significantly higher during infection. There were no significant differences for the other parameters. Positive correlations between haptoglobin and total protein ($r = 0.434$), haptoglobin and albumin ($r = 0.409$) and CRP and lymphocytes ($r = 0.422$) were found. The results obtained for the dogs suggest a chronic inflammation during the kidney parasite infection.

IMMUNOHISTOCHEMICAL TECHNIQUES FOR DIAGNOSIS OF GM1 AND GM2 GANGLIOSIDOSES IN DOGS AND CATS

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GM1 and GM2 gangliosidoses are progressive neurodegenerative lysosomal storage diseases resulting mainly from the excessive accumulation of GM1 and GM2 gangliosides in lysosomes, respectively. These diseases are inherited in an autosomal recessive manner resulting in the premature death of affected individuals due to brain damage with progressive neurological signs. The diagnosis of these diseases are based on comprehensive findings, which include clinical, biochemical, histopathological, and genetical factors using various types of specimens.
Therefore, in some cases, individuals have been diagnosed incorrectly resulting from insufficient specimens. This study aims at establishing immunohistochemical techniques for the diagnosis of canine and feline gangliosidoses using only paraffin-embedded sections.
Paraffin-embedded brain samples from Shiba Inu dogs and domestic cats with GM1 gangliosidosis, a mix-breed dog and domestic cats with GM2 gangliosidosis, and some controls were used in this study. Immunohistochemical analysis was performed using biotinconjugated cholera toxin B subunit (List Biological Laboratories, Inc.) for detection of GM1 gangliosides, and biotin-conjugated mouse monoclonal antibody against N-acetyl GM2 ganglioside (Tokyo Kasei Kogyo, Co., Ltd.) for detection of GM2 gangliosides. Peroxidaselabeled streptavidin and 3,3'-diaminobenzidine, a peroxidase substrate, were used for the visualization of samples. The immunohistochemical staining demonstrated the GM1 and GM2 gangliosides, from samples of GM1 and GM2 gangliosidoses, respectively, were accumulated in the cytoplasm of swollen neurons in the brains. Therefore, the immunohistochemical techniques are reliable tools for the diagnosis of canine and feline gangliosidoses when only paraffin-embedded blocks are available. Moreover, these techniques can be used to examine past cases of suspected gangliosidosis if a paraffin-embedded block of the brain is available.

PROGNOSTIC VALUE OF IMMUNOCYTOCHEMISTRY IN CANINE LYMPHOMA

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The purpose of this study was to evaluate expression of proliferation and survival markers in lymph node aspirates from dogs with lymphoma using immunocytochemistry (ICC) to determine if differences existed that could improve prognostic capability. The approach is inexpensive, minimally invasive, rapid, and readily accepted by clients.

The proteins Ki 67 and survivin, are markers of cellular proliferation and survival, respectively, and their over-expression in human and canine lymphomas using surgical biopsies and immunohistochemistry has been demonstrated.

Thirty dogs diagnosed with lymphoma were staged and all received chemotherapy selected on the basis of cytological features of node aspirates. ICC for survivin, Ki67, and B and T cell markers was performed on lymph node aspirates taken prior to treatment and slides were evaluated for relative expression of these markers. Remission status was monitored clinically and nodes re-aspirated for cytological evaluation and ICC for markers at relapse or at the conclusion of the study if still in remission. Dogs were classified as early relapsers (< 8 week) or late relapsers (> 8 weeks).

The percentage of Ki67 positive cells prior to treatment was significantly higher for early vs late relapsers. Low initial Ki67 scores correlated with longer survival. Survivin expression did not differ between early and late relapsers. No difference in expression of either marker between B and T cell lymphomas was found.

Evaluation of Ki 67 expression but not survivin by ICC on lymph node aspirates may improve prognostic capability for canine lymphoma patients.

COMPARISON OF THE PATTERNS OF MILK SERUM PROTEINS IN SUBCLINICAL MASTITIS CAUSED BY FOUR COMMON PATHOGENS IN DAIRY COWS

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Acute phase proteins (APPs) are group of proteins that their increase (positive acute phase proteins) or decrease (negative acute phase proteins) can be a sign of inflammation, trauma, or infections in the mammary glands. Those APPs in serum and milk which are separable by cellulose acetate electrophoresis include albumin, immunoglobulin, α-lactalbomin and β-lactoglobulin. The objective of this study was to compare the increase or decrease patterns of serum milk APPs during subclinical mastitis caused by S.agalactiae, S.aureus, S.uberis and C.bovis and also to determine the correlation coefficients of these proteins with somatic cell counts as the gold standard of subclinical mastitis.

From February to October 2010, 45 Holstein cows were selected randomly from 5 dairy farms in Tehran province, Iran. 11 quarters with S. agalactiae, 11 quarters with S. uberis, 12 quarters with S. aureus, and 11 quarters with C. bovis were cultured.

The bacteriologic results from the cows in this study with subclinical mastitis reflected the usual pathogenic bacteria, S. agalactiae and S. aureus, isolated from quarters affected with subclinical mastitis in Iran.

The results of the present study showed that the studied pathogens caused different electrophoretic patterns in milk of the affected quarters. So it can be concluded that the electrophoresis of serum milk samples could be used to differentiate the pathogens causing subclinical mastitis.

VETSCAN i-STAT 1 - COMPARISON OF MEASUREMENTS OF SOME VARIABLES WITH THE ROUTINELY USED LABORATORY METHODS

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VetScan i-STAT 1 (Abaxis) is a portable handheld analyzer for measuring different biochemical and some haematological variables in blood. The aim of our research was to compare measurements on analyser VetScan i-STAT 1 with methods routinely used in clinical laboratory performed on biochemical analyser RX Daytona (Randox) and haematological analyser ScilVet abc Plus (Horiba). Both routinely used analysers are included in internal and external international quality control. The measurements were performed in 18 blood samples of cows. We compared the results for glucose, sodium, potassium, chloride, urea, creatinine, haematocrit and haemoglobin.

The mean values and standard deviations for investigated variables and methods used were calculated. The correlation coefficients were 0.948 for sodium, 0.908 for chloride, 0.977 for urea, 0.939 for creatinine, 0.939 for haematocrit and 0,940 for haemoglobin, 0.839 for glucose and 0.831 for potassium. The calculation of the agreement confirmed that, on average, there is good agreement between VetScan i-STAT 1 and routinely used method for glucose, sodium and chloride. For other variables VetScan i-STAT 1 gave on average lower results than routinely used methods namely; 0.43±0.20 mmol/L for potassium, 0.65±0.34 mmol/L for urea, 13.05±5.90 µmol/L for creatinine, 4.07±1.34 % for haematocrit and 1.41±0.34 g/dl for haemoglobin.

Our results revealed that differences between VetScan i-STAT 1 and routinely used methods for potassium, urea, creatinine, haematocrit and haemoglobin are clinically important. This should be considered when interpreting results or reference intervals should be adjusted for VetScan i-STAT 1.

HAEMOLYTIC ANAEMIA IN HORSES AND DONKEYS

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Poisoning of horses with plants is rare in Slovenia because hay is obtained mainly from fertilized meadows where herbal diversity is small. Nevertheless, blood samples from a mare were submitted to our laboratory with history that one horse died suddenly and that the mare is anorexic and lethargic. The owner also has two donkeys, a male and a pregnant female that were also depressed. Haematology revealed marked anaemia and leukocytosis with neutrophilia (RBC 2.31 x1012/L, MCV 42 fL, Hb 5.8 g/dL Ht 9.7 %, WBC18.9 x 109/L). Biochemical analyses indicated severe haemolytic icterus and liver damage (bilirubin 237.35 µmol/L, AST 1283 U/L, GGT 81 U/L and ALT 130 U/L). Haemolysis, agglutination and destruction of red blood cells were observed in blood smears, which made morphological changes very difficult to evaluate.We suspected poisoning. Examination of the hay that was fed to the animals for two weeks revealed a large amount of poisonous herb mouse garlic, Allium angulosum, which contains N-propyl disulfide, a strong oxidant. Despite symptomatic therapy the mare died next day. Since donkeys ate the same food, we recommended examination of their blood as well. Results of the blood samples showed marked anemia, especially in the male (RBC 1.64 x1012/L, MCV 59 fL, Hb 5.2 g/dL Ht 9.7 %, WBC 21.9 x 109/L). Blood smears revealed presence of Heinz bodies in almost every RBC, which are typical for oxidative injuries. Donkeys were symptomatically treated with iron and B vitamin and they both survived. The ingestion of large quantities of Allium angulosum caused severe, potentially fatal haemolytic anaemia due to oxidative damage of erythrocytes in horses and donkeys.

TISSUE FACTOR, PLASMINOGEN AND PLASMINOGEN ACTIVATOR INHIBITOR IN DOGS WITH GASTRIC DILATATION AND VOLVULUS

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Gastric dilatation and volvulus syndrome (GDV) is frequently associated with alteration in coagulation and fibrinolytic system leading to disseminated intravascular coagulation (DIC). However, trigger of DIC in these dogs is not known yet. Aim of this study was to compare level of tissue factor (TF), plasminogen (P) and plasminogen activator inhibitor (PAI) in dogs with GDV and healthy controls and to correlate severity of haemostatic imbalance with these parameters. Platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), level of fibrinogen (FBG) and D-dimers, TF, P and PAI were measured in 19 dogs with GDV and 12 healthy dogs. In dogs with GDV, alteration of each PLT, PT, aPTT, FBG and D-dimers was scored by 1 point. Levels of TF, P and PAI were compared between GDV and control dogs using Mann-Whitney U test, correlation of score and TF, P and PAI was performed using Spearman correlation coefficient. Significant difference between control dogs and dogs with GDV was found in PLT (259 vs. 179x109/l, $p=0.01$), PT (9.7 vs. 10.9 s, $p<0.0001$), D-dimers (0.1 vs. 0.4 mg/l, $p<0.01$) and PAI (31.6 vs. 52.4 pg/ml, $p<0.05$). TF (median both below detection limit) and P (37.6 vs. 11.6 ng/ml) did not differ significantly. There was no correlation between score and TF, P or PAI. Our results indicate higher activity of plasminogen activator inhibitor in dogs with GDV what may contribute to development of DIC in these patients.

CHANGE OF ANTIBODY LEVELS TO FERRITIN IN THE SERA OF FOALS AFTER BIRTH: POSSIBLE PASSIVE TRANSFER OF MATERNAL ANTI-FERRITIN AUTOANTIBODY VIA COLOSTRUM AND AGE-RELATED ANTI-FERRITIN AUTOANTIBODY PRODUCTION

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In mammals, ferritin-binding proteins are involved in ferritin clearance. Mammalian antiferritin autoantibody is known as a common ferritin-binding protein. However, it remains to clarify the development and class change of anti-ferritin autoantibody. The aim of this study is to examine changes of antibody (IgG, IgM or IgA) levels against ferritin in the serum of foals after birth by semiquantative measurement with normalization with each antibody activity against the ferritin in reference adult horse serum. After addition of the ferritin to serum samples, complex formed between the ferritin and antibodies to ferritin in these samples was immunocoprecipitated using antibody to horse ferritin. Antibody classes in the immnocoprecitate were detected with antibodies specific for each immunoglobulin. Seven adult horse serum samples were found to have ferritin-binding activities in all immunoglobulin classes. Relative activities of antibody classes against the ferritin in six foal sera (3 males and 3 females) on day 1 and at 2, 10, 20, 28, 36, 40, 52, and 56 weeks after birth were semiquantatively determined with ferritin-binding activity of each antibody class of a reference serum. IgG and IgA antibody activities to ferritin were scant in the sera on day 1, while IgM antibody binding activity was faintly detected. Although antibody activities against ferritin of IgG and IgA showed a biphasic pattern after birth, those of IgM showed the tendency to increase. Higher antibody (IgG, IgM, and IgA) activities to ferritin were detected in colostrums. These results demonstrate that antibody to ferritin in foal is derived from maternal colostrum just after birth and is produced thereafter.

NOTES

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